THESIS

# THE USE OF COMPUTER ASSISTED SEMEN ANALYSIS TO PREDICT FERTILITY IN HOLSTEIN BULLS

Submitted by

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ABSTRACT

THE USE OF COMPUTER ASSISTED SEMEN ANALYSIS TO PREDICT FERTILITY IN HOLSTEIN BULLS

Cryo-preserved semen from 120 Holstein bulls was obtained from three semen companies, which were randomly coded 1:3 to decrease bias, through USDA-ARS, Fort Collins, CO. Computer assisted semen analysis (CASA; HTM-IVOS, Version 10.8, Hamilton Thorne Research, Beverly, MA, USA) was used to assess seminal characteristics and to determine if this was useful for predicting fertility, defined in this study as sire conception rate (SCR). For this study the primary CASA measurements assessed were percent motility, percent progressive, average pathway velocity (VAP), straightline velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN) and cell size (SIZE). Percent motile and percent progressive sperm cells had means, followed by their standard deviation, of 60.93 ± 10.09 and 32.46 ± 10.06%, respectively with percent motile having the lowest coefficient of variation of 16.72 %. Amplitude of lateral head displacement and BCF were both high when compared to other studies conducted with fresh and cryopreserved semen (Budworth et al.,1988, Farrell et al., 1998) with means, followed by their standard deviation, of 8.45 ± 3.5μm and 30.99 ± 9.27Hz, respectively. Straightness and LIN had means, followed by their standard deviation of

80.74 ± 17.11 and 48.24 ± 16.15%, respectively; SIZE had a mean, followed by its standard deviation, of 8.38 ± 2.88μm$^2$. After initial data exploration the data was fit either untransformed, or transformed. Data was fit untransformed because of the data being normally distributed or because a cubic model fit best. Data was fit transformed to better fit normality requirements, for prediction of CASA values. SIZE appeared to have a cubic relationship with all three velocity parameters (VAP, VCL and VSL). The number of bull samples originating from each semen company is as follows: Semen Company 1(SC1) - 32, Semen Company 2(SC2) -71, and Semen Company 3 (SC3) -17. Semen company, when fit as a fixed effect was found to be significant ($P \leq 0.05$) so this study looked at the differences of the least squares means between semen companies. When looking at untransformed data SC1 and SC2 were significantly ($P \leq 0.001$) different across VAP, ALH and BCF. The heritability of most seminal parameters was low, with the exception of percent motile (0.793). Percent motile also had the greatest genetic variance when compared to its residual variance. Genetic correlation of VAP, ALH and SIZE with SCR were low (0.05, 0.006, and 0.04 respectively); however, percent motile was moderately genetically correlated with SCR at 0.302. After VAP and ALH were transformed to meet normality requirements, heritability was once again calculated and the heritability of the transformed data was lower than the untransformed data. The genetic correlations between the transformed data and SCR stayed the same (0.052) or improved (0.02) ($\log_{10}$VAP and $\sqrt{}$ALH, respectively). The low heritabilities of these seminal traits does not make them good candidates for genetic evaluation. Significant differences between semen companies suggest that the methods semen companies use to select bulls, collect semen from bulls, extend semen and store the cryopreserved semen

could be different. However, this information is highly proprietary and is difficult to ascertain to truly attribute difference between semen companies. Due to the genetic correlations between CASA values and SCR being low we were unable to use this study's CASA values for genetic prediction of fertility. While CASA values do have an underlying genetic component, environmental effects are too large and physiological processes vary too much to estimate future performance of these bulls' fertility.

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*I would like to dedicate this thesis to my mom, dad and husband. All three of you have loved me and been patient with me through everything.*

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# INTRODUCTION

Bull fertility plays a very important role in bovine reproduction since selection intensity of bulls chosen for breeding programs in both the beef and dairy industry is high and since they can produce high numbers of offspring relative to females. Bulls that are sub-fertile prolong calving season due to delayed pregnancy, reducing calf crop weights and in turn increasing culling of females (Kastelic and Thundathil, 2008).

The problems created by sub-fertile or infertile bulls are of economic importance to the cow-calf producer. As a general category, reproduction is at least five times more important economically than growth traits to the beef cow-calf producer (Trenkle and Willham, 1977). Any measurable trait that is able to identify lower fertility bulls to cull or avoid, or conversely highly fertile bulls to utilize in a herd should improve the fertility rate of a herd (Clay and McDaniel, 2001). In the dairy industry this allows a cow to begin lactation sooner and in the beef industry this provides an opportunity for the cow to produce a heavier calf at weaning.

Reproductive performance and fertility in beef bulls is influenced by many different factors, including testicular development, seminal quality, libido, mating ability and physical soundness (Ott, 1986; Chenoweth, 1983; Larson, 1986). Traditionally breeding soundness exams are used to predict whether a bull will likely be a good breeder or not. Additionally, many studies have focused on seminal quality, using two main methodologies: trained personnel using a microscope or more recently computer assisted semen analysis (CASA). The CASA system allows for a more objective measurement of

semen quality than traditional methodologies. However, there are limitations on the use of this machine due to cost; wide variability in equipment settings due to the flexibility in machine parameterization as well biological limitations; as the fact that many sperm attributes are required for sperm to fertilize an oocyte, not merely motility, and since lab assays generally score sperm populations not individual sperm (Rodríguez-Martínez, 2006; Rodríguez-Martínez, 2007).

Even with all of the technology available there are differing ideas on which methods best predict bull fertility, and which methods best fit the breeding scheme of any given ranch. While people agree that certain correlations exist between fertility and sperm parameters the problem is these are not consistent between studies. There is little consensus on what parameters can be used to build an adequate model for predicting fertility and the problem arises that fertility is defined so many way, conception rate, pregnancy rate, calving rate. Few studies have been conducted to analyze the association of seminal quality with fertility and look at the genetics behind seminal quality. Of the studies that have been conducted, most deal with poultry and are limited to a few genetic lines. There are few studies published on the genetics of fertility in cattle, specifically with regards to seminal characteristics.

The objectives of this study are to:

- Determine if there is a genetic component to CASA parameters, and
- Predict fertility, defined as sire conception rate, using CASA parameters.

# LITERATURE REVIEW

## *Fertility*

Bull fertility is essential to reproductive performance; bulls have a high selection intensity, therefore, fertility is of the utmost importance. The Beef Improvement Federation (BIF) guidelines estimates around 20% of beef bulls have some degree of infertility (2010). Fertility is import to cow-calf producers so that they are able to provide themselves and others with herd replacements. In the dairy industry fertility is essential to milk production, if a bull is in-fertile, in the case of natural mating, or sub-fertile, in the case of artificial insemination, and the cow isn't bred back it is unable to enter lactation again and thus unable to produce.

Fertility has been defined many ways. A common definition of fertility is a set nonreturn rate where if the cow hasn't returned to heat or been rebred within a specified number of days, they are considered pregnant. Some examples of these day limits are a 70 d nonreturn rate (Clay and McDaniel, 2001; Kuhn and Hutchison, 2008), a 75 d nonreturn rate (Budworth et al., 1988; Budworth 1987), and a 59 d nonreturn rate (Farrell et al., 1998). Budworth (1987) and Budworth et al. (1988) based fertility on the percentage of cows and heifers that were apparently pregnant at 75 d after insemination. Farrell et al. (1988) defined fertility as the 59 d nonreturn rate to first service. Clay and McDaniel (2001) as well as Kuhn and Hutchison (2008) utilized a 70 d non-return rate because of its use in calculating Estimated Relative Conception Rate.

Another way to determine pregnancy is by palpation or ultrasound *per rectum*. Farin (1980) used palpation *per rectum* at 60-75 d post insemination to diagnose pregnancy and in return determine fertility.

Budworth et al. (1987) used an alternative method, a competitive fertility index, which was calculated using phenotypic markers and bloodtyping to determine sire of calves. Equal numbers of sperm from two bulls were mixed into one dose and every cow was inseminated with one mix. This is a relative fertility estimate because of the heterospermic insemination, one bull was a success and one bull was a failure. These bulls were ranked according to their competitive fertility (Budworth et al., 1987).

Fertility in chickens is defined differently because of their different nature of laying eggs. In chickens candling (shining a bright light through the egg) eggs after 7 d of incubation was used to determine fertility from hens that lay 2 or more settable eggs (Ansah and Buckland, 1983). The BIF uses the stayability EPD as a prediction of sustained female fertility, but as of now has no set definition for immediate fertility (Beef Improvement Federation Guidelines, 2010).

Farin (1980) analyzed data by least squares analysis and discovered 7 different models to assess libido/serving capacity and mating/fertility performance in a 30 hr observation period. The variables analyzed were trial (1-6), age of bull, libido, number observed in estrus (linear), observation time (hr, linear & quadratic), number services per female (linear), and last service number to each female (linear). Model 2 analyzed measurements of mating performance and fertility during the 30 hr observation period. Model 4 analyzed the effect of successive services on number pregnant. In model 2 the number in estrus, trial x libido, and age of bull x libido were found nonsignificant. In

model 4, bull libido, BSE score, percentage of total abnormal sperm cells, normal sperm cells, and motile cells, rate of mobility, scrotal circumference, age of bull by scrotal circumference and mean services per female were all found to be non-significant main effects. With the amount of fertility related information available, to select a complete model that accurately predicts fertility, the genetics, or the pedigree, should be included.

*Genetics of Fertility*

While many studies have been done to assess the correlation between seminal characteristics and fertility in animals and humans (Bailey et al., 1994; Branton et al.,1950; Kaskar and Franken, 1996), few studies have addressed the genetics of fertility. Ansah and Buckland (1983) examined the effect of selection on four aspects of fertility of frozen-thawed and fresh semen in chickens. Eggs were used as the determinant of fertility by candling them after 7 d of incubation from hens that laid 2 or more settable eggs. They calculated the heritability and repeatability of the estimates of fertility and hatchability of the eggs from frozen-thawed semen. The authors found the heritability of fertility in their selected line ($h^2 = 0.01$) was lower than the heritability of fertility in the control line ($h^2 = 0.31$), and attributed this to non-random mating or selection for semen that survived better in the freezing process. However, the selected line expressed greater fertility when compared to the control line. Ansah et al. (1985) continued with the breeding lines started in the previously mentioned paper and analyzed the effect fertility selection had on seminal characteristics. This study also utilized the candling of eggs after 7 d of incubation from hens that laid 2 or more settable eggs as the determinant of fertility. They found a significant difference between the selected line and the control line, when analyzing fertility of frozen-thawed semen. The result was a higher level of fertility and

duration of fertility (defined as days semen is frozen prior to insemination) in the selected line than in the control line. When looking at the seminal characteristics, the authors found that selecting for fertility of frozen-thawed semen also decreased the seminal fluid volume and sperm number per ejaculate, but had no effect on sperm concentration. While the quantity of the sperm decreased over time, the quality of the sperm increased. The selected line had significantly fewer abnormal sperm present and a significantly higher percentage of normal sperm when compared to the control line. There is a favorable genetic association between semen production and egg production (Nestor, 1977, Marks, 1978). Because of this, Buckland's group assessed the genetics of sperm/semen characteristics and egg weight. They found low to moderate genetic correlations of between rooster semen weight (0.19, 0.20), packed-sperm volume (0.26, 0.07), and total sperm weight (0.36, 0.24) with egg weight at both d 240 and 450 in their selected line with the control lines having varying genetic correlations (respectively; Segura et al., 1990). When the control lines' correlations were averaged across the lines, Full-sib and Dam-son, they were in the same range as the selected strain. Genetic correlations with egg weight at both day 240 and 450 were as follows: semen weight (0.20, 0.26), packed-sperm volume (0.06, 0.05), and total sperm weight (0.16, 0.18; Segura et al., 1990).

Ansah et al. (1985) found that the heritabilities and repeatabilities of ejaculate volume, sperm concentration, and sperm cells per ejaculate were much higher for the control line than the selected line. However, the difference in repeatability for each line was not as great as the difference in heritability values. Heritabilities for the selected and control lines, respectively, are as follows: ejaculate volume (0.34, 0.64), sperm concentration (0.37, 0.65), and sperm cells per ejaculate (0.54, 0.73). Repeatability for

the selected and control lines, respectively, are as follows: ejaculate volume (0.35, 0.48), sperm concentration (0.33, 0.50), and sperm cells per ejaculate (0.42, 0.51). The authors attributed the lower heritability in the selected line to decreased genetic variance caused by a greater selection pressure. Segura et al. (1990) also found the heritabilities of the traits they assessed to be lower in the selected line than in the control line. The authors concluded that wattle and testes weights are influenced mainly by additive genes because of the large heritability estimates ($0.54 \pm 0.10$ and $0.58 \pm 0.11$). The authors concluded that semen volume and sperm production of males are not good indicators of high egg production in female relatives because of the unfavorable genetic relationship between semen traits and egg production traits These egg production traits included 1) Age at first egg, 2) Hen-day rate of egg production 3) The number of eggs produced from housing to two different ages 4) Egg weight 5) Egg specific gravity, and indicator of shell thickness 6) Albumin height 7) Shell shape and 8) Blood spots percentage. These can be compared to mammals traits such as, age at puberty, calving rate, the weight of calf to different weaning days, and calf birth weight.While the previous findings highlights testes weight as a poor indicator of fertility, in cattle scrotal circumference is correlated with daughter age at first puberty.

*Scrotal Circumference*

Scrotal circumference (SC) has been found to be favorably related to semen quality as far back as the fifties (Bratton et al., 1956; Bourdon and Brinks, 1986). Scrotal circumference is highly heritable and positively correlated to total semen production, semen quality, and testicular weight (Latimer et al., 1982, Hopkins and Spitzer, 1997). Heritabilities in the literature for SC of yearlings, unadjusted, are $0.6 \pm 0.17$ (Latimer et

al., 1982), 0.36 ± 0.06 (Knights et al., 1984) and 0.53 ± 0.06 (Bourdon and Brinks, 1986). Coulter et al. (1976) found the weighted average for heritability of SC to be 0.67 and the correlation of SC with spermatozoal output to be 0.81. Since SC is highly heritable, producers have put great selection pressure on larger scrotal sizes. This allows producers to breed heifers sooner thereby decreasing the length of the calving season, which helps to produce bulls that meet the standards for Breeding Soundness Exams (BSE). The Society for Theriogenology (Chenoweth et al., 1992) has published minimum guidelines for scrotal circumference for different age ranges (Table 1) in an effort to encourage genetic progress in breeding cattle.

Table 1. Minimum scrotal circumference by age [a]

| Age | SC (cm) |
|---|---|
| ≤ 15 months | 30 |
| > 15 to ≤ 18 months | 31 |
| > 18 to ≤ 21 months | 32 |
| > 21 to ≤ 24 months | 33 |
| > 24 months | 34 |

[a] Adapted from Chenoweth et al., 1992

Madrid et al. (1988) attributed poor quality semen to small SC measurements. A SC of less than 32 cm was considered small. The only difference found in a study done by Chacón (2001) between Zebu bulls older than 24 mo with a SC of ≤ 30 cm and bulls whose SC > 30 cm was a higher prevalence of cytoplasmic droplets. This may be the result of a delayed sexual maturity or a disruption in the sperm maturation process (Chacón, 2001).

Several studies have evaluated the ability to predict SC at later ages using early life measures. Coe and Gibson (1993) evaluated bulls at 200 d and 365 d and found that calves with > 23 cm SC at 200 d had a 95% probability of achieving > 34 cm SC by 365 d. Decker et al. (2008) found that an adjusted 240 d SC measure of 22.5 cm was required

to achieve 30 cm SC at 365 d. This is more desirable information for producers who are able to choose bulls at an earlier age for development, and provides a sense of assurance that the chosen bulls are able to pass their BSE to either be used in the herd or sold for profit.

*Breeding Soundness Exam*

Breeding soundness exams are widely used and commonly accepted for predicting bull fertility (Kealey et al., 2004). The Society for Theriogenology lists three classifications for bulls as a result of their BSE (Chenoweth et al., 1992). These three classifications are 'satisfactory potential breeder', 'unsatisfactory potential breeder', or 'classification deferred' for re-testing later. To be classified as a 'satisfactory potential breeder' the bull must have a good physical evaluation, a minimum scrotal circumference based on age (Table 1), a minimum of 30% individual motility, and a minimum of 70% normal morphology.

It is very common for yearling bulls to have a less than satisfactory SC, or have less than satisfactory sperm morphology, or both. Makarechean and Farid (1985) found yearling bulls to have a greater frequency of primary semen abnormalities (14.6%) and lower scrotal circumference (35.2 cm) than mature bulls (10.2% and 38.7 cm, respectively). Kennedy et al. (2002) found a greater percentage of 10 mo old bulls being classified as unsatisfactory due to inadequate SC than 17 and 18 mo old bulls (24.9% and ~8.6%, respectively). Primary semen abnormalities have a greater effect on fertility than secondary abnormalities, however, some indicate a lack of maturity, and disappear after the bull has matured; in this case the bull would be classified 'classification deferred'. Another main reason 'classification deferred' is used is when bulls have a temporary

injury or illness that when healed would allow the bull to satisfactorily pass the BSE. When classifying bulls the evaluator has set guidelines for what is considered a satisfactory potential breeder, however once a bull doesn't meet all the requirements the evaluator decides whether to classify as 'unsatisfactory potential breeder', or as 'classification deferred'. While this method is highly accepted and highly utilized it is very subjective, requires the use of highly trained personnel, and BSE score is lowly heritable, 0.10 ± 0.06 (Smith et al., 1989).

A current ongoing discussion amongst animal breeding and genetics researchers is whether failing a bull on his BSE for not having a 30 cm scrotal circumference is fully justified. One argument commonly used could be stated as, "If a bull's semen is viable and he is able to perform, with a scrotal circumference less than 30 cm is it justified to consider him an unsatisfactory potential breeder?" While many studies have been done to justify a cutoff of 30 cm, there are breed differences that need to be assessed when performing a BSE. Sosa et al. (2002) found the SC of Wagyu bulls to be smaller than Angus and Brahman bulls at puberty, 24.5 ± 0.8 cm, 28.1 ± 0.8 cm and 28.0 ± 0.7, respectively. Kennedy et al. (2002) also found a significant difference between the percent of bulls within breed classifying as unsatisfactory due to SC. Angus had only 4.8% with an inadequate SC while Limousin, Santa Gertrudis, and Simbrah were all greater, 16.8 ± 4.5%, 35.8 ± 2.8%, and 22.4 ± 4.7%, respectively. The differences between breeds are visible in carcass and fertility traits and as such, breeds are selected for their respective desired traits. A basic breakdown of British, European and Zebu breeds is shown in Table 2. British breeds are usually selected for their maternal traits, low birth weight, and higher fertility. European breeds are usually chosen for their

finishing traits, high growth weight and larger frame size. Zebu breeds are chosen for their adaptability to adverse environments such as, high heat, low nutrition, and high insects. Since this is the case, why do all breeds have the same SC requirements? Why not consider culling at the level of any bull that is greater than one standard deviation from their breed mean for SC? While this may decrease genetic variation, there is potential for less of a decrease as compared to a culling level of 30 cm.

Table 2. Advantages and disadvantages to different breed types.[a]

| Breed Type | Advantages | Disadvantages |
|---|---|---|
| **British** | | |
| Angus, Hereford, Shorthorn | • Low birth weight<br>• High fertility<br>• Moderate milk production<br>• Moderate frame size | • Lower growth rate |
| **European** | | |
| Charolais, Gelbvieh, Maine-Anjou, Pinzaguer, Simmental, South Devon, Tarentaise, and Others | • High growth rate<br>• Larger frame size<br>• High milk production | • Increased dystocia<br>• Increased maintenance |
| **Zebu** | | |
| American Brahman, British Derivatives, and European Derivatives | • Adaptability to adverse environments<br>• Moderate to high milk production<br>• Maximum heterosis in crossbreeding | • Lower growth rate<br>• Poor carcass quality |

[a]Adapted from Hicks et al., 2010

Hopkins and Spitzer (1997) outlined proper collection of SC measurements. First testicles should be gently massaged to the bottom of the scrotal sack. The measuring tape should then be placed over the area of greatest width and the tape pulled until snug. This presents another problem with the subjectivity of BSE. While evaluators are highly trained, each has their own degree of 'snug' for the measuring tape that can make the difference between a 29 and a 30 cm SC. This can cause problems with yearling bulls

who may meet all minimum requirements but the SC measurement. Kastelic and Thundathil (2008) concluded that while traditional BSE may identify bulls that are abnormal, a comprehensive approach of assessing sperm function and fertility at the molecular, cellular and whole-animal levels is necessary to predict fertility of bulls producing normal sperm. Bruner et al. (1995) performed BSE and analyzed the difference between including and excluding semen analysis and how bulls are classified. When semen analysis was included less bulls were classified as 'unsatisfactory', but more were classified as 'questionable' or 'classification deferred'. They attributed this to the fact that primary abnormalities are the most significant factor in determining BSE classification. BSE should be a portion of a bull's assessment concurrently with seminal evaluation to maximize breeding potential.

*Computer Assisted Semen Analysis*

Computer assisted semen analysis (CASA); also known as sperm quality analyzer, computer assisted sperm motility analysis, and a microcomputer-photographic method for evaluation; has been used for many years to provide an objective measure of semen characteristics. Until recently the ability of these machines to correctly analyze a semen sample and in turn predict fertility was poor and subject to human error. However, there has been a push for machines that not only estimate fertility, but accurately estimate fertility because it has been estimated that breeders would pay a premium of $2 for semen they purchase for each 1% increase in fertility rates (Clay and McDaniel, 2001). The most recent CASA machines are still not 100 % objective because of the bias in operators setting limits on what is considered motile, and what is considered debris.

> "CASA and visual measures of percent motility usually differ because their definitions are not identical. In visual measures, a spermatozoon is usually considered to be motile if its flagellum is twitching even though it may not exhibit forward progression. In CASA, a spermatozoon must achieve a minimum VSL to be motile," (Davis and Katz, 1996).

Budworth et al. (1987) and Farrell et al. (1997) set a threshold level of velocity for sperm to be considered motile at $\geq 20$ μm/s.

Palmer and Barth (2003) reported disadvantages of the CASA machine, in this instance the Optibreed™, BullMate™ sperm quality analyzer (Alpharma Inc., Fort Lee, NJ) to be high cost and difficult to calibrate, validate and standardize. There are other disadvantages to the CASA system, but many of them are easily overcome by appropriate parameterization of the machine. Below is a list presented by Davis and Katz (1996) of factors that can affect CASA results:

- Instrument precision
- Instrument accuracy
- Microscope
- Counting chamber
- Diluents or extender
- Video framing (or digitization) rate
- Physiological state of sperm
- Temperature effect on VCL and motility
- Specimen concentration
- Presence of debris in the sample
- Instrument parameter settings
- Digitization threshold (gray scale)
- Number of points per track
- Number of frames tracked
- Number of fields (or sperm) analyzed
- Computational algorithms for average path and ALH
- Statistical methods
- Laboratory supplies
- Videotaping (inter-tech variation)
- Between-aliquot variation

Many of these can be overcome by simple solutions such as, using pre-warmed slides from the same company, maintaining an average number of cells viewed per frame, setting the minimum and maximum parameters on the machine for clarity, and eliminating cells that aren't alive, moving, or swim off the screen as soon as the analysis starts.

With the availability of different slide choices on the market, such as 2-chamber, 4-chamber, and 8-chamber slides; it is wise to first determine what best suits the machine being used, which can easily be accomplished by consulting the operations manual. Then continuing with the same slide throughout the study will decrease variability due to slide differentiations. Pre-warming slides allows them to be at the same temperature the semen samples are at so the samples don't experience a temperature shock. Machines that have been used to analyze sperm have also been checked for repeatability by using more than one slide of the same sperm sample. Initially Budworth et al. (1987) found that variation in the percent of motile sperm decreased when two or three slides were used rather than one slide. Later Budworth et al. (1988) found that slide to slide variation was low but suggested that two slides be used to reduce error.

Alleviating issues associated with debris in the sample has proven problematic. Budworth et al. (1988) found that a range of 0 to 18% of approximately 240 spermatozoa identified might be debris from the extender. The authors then suggested that for semen to be properly evaluated using a computerized system; particulate matter in an egg yolk extender must be reduced by sedimentation over night at 5 C followed by decantation, centrifugation, or filtration. Extender is not always known when samples are analyzed by

the CASA machine so operators should take great care in observing the amount of debris present in the field of view.

When it comes to fertility Farrell et al. (1998) reported significant, high correlations (0.99) between bull fertility, 59 d non-return rate to first service, and CASA motility parameters; BCF, linearity, average path velocity, straightness, curvilinear velocity, total motility, linearly motile sperm, and total number of motile sperm. Kasimanickam et al. (2006) found ALH and BCF together with either VSL or VAP to be significantly correlated to fertility which they defined by using a competitive fertility index. Computer assisted semen analysis has the potential to more accurately predict fertility than traditional BSE and visual evaluations (Farrell et al., 1998; Christensen et al., 1999). Computer assisted semen analysis is a broad term that is used for computerized semen analysis and there are machines that are currently being used under different names and have been reported in studies to be good estimators of sperm viability.

*Other Objective Measures*

Kruger and du Toit (1996) report the use of automated sperm morphology analysis (ASMA). Automated sperm morphology analysis machines work like CASA machines except that no movement parameters are recorded and the slide is fixed and stained. The set up consists of a microscope, a video camera, a computer frame grabber and morphology software. The computer frame grabber receives images from the video camera for analysis by the morphology software. This software uses many of the same parameters as CASA for sperm recognition, size, shape, intensity, and others. When debris in the sample are sorted out by the computer, the sperm head, midpiece, acrosome,

and others are measured metrically. These measurements are used to assign the sperm to different classifications; normal, subnormal, or abnormal. The correlation of the computerized machine with manual inspection of morphology was positive and favorable (Kruger and du Toit, 1996).

Mahmoud et al. (1998) reported the use of the sperm quality analyzer (SQA) as a portable device for fast evaluation of semen quality; it requires minimal training and is considered relatively cheap when compared to other CASA systems. The output of the SQA is a value expressed in sperm motility index (SMI) units. These are a measure of the optical density fluctuations caused by motile cells. Zavos et al. (1996) better described SMI as reflecting semen concentration, morphology and acrosomal status of motile spermatozoa. Palmer and Barth (2003) stated that there was a threshold SMI value of 350 where bulls would have a satisfactory BSE. They continue to suggest that the SMI should be referred to as the sperm quality index, because it assesses more than just motility. The authors also reported a problem with the SMI being a single output, the index value; semen samples with one or two poor qualities (i.e. low percent motile sperm) may go undetected because the good characteristics of the sample would conceal the failing characteristics.

Plasma membrane status or integrity (PMI) has become of greater importance due to the effect cryopreservation has on sperm cell membranes. The plasma membrane is the outermost membrane on the head of the spermatozoon, followed by the acrosomal membrane and the nuclear envelope; a mitochondrial membrane covers the midpiece of the spermatozoon (Kasimanickam et al., 2006). Graham et al. (1990) stated that sperm competency requires that each of these membranes be intact, without this, fertility will be

compromised. When assessing PMI a combination stain of SYBR® 14 or CDMFDA (carboxydimethylfluorescein diacetate) and propidium iodide (PI) are used for labeling the sperm cell as live or dead. SYBR® 14 is a membrane permeable, nucleic acid stain that penetrates the intact plasma membrane and stains the DNA green, while PI is membrane impermeable and stains the DNA red only when the plasma membrane is damaged (Ericsson et al., 1993; Kasimanickam et al., 2006).

Kasimanickam et al. (2006) chose to evaluate the relationship of PMI and sperm motility parameters (using CASA) and found a significant, positive correlation ($r = 0.87$, $P < 0.01$) between PMI and the total progressive motility assessed by CASA. The same correlation was found between the PMI and the competitive index formed in the study conducted by these authors, however, it should be noted that the PMI along with the DNA fragmentation index were used to calculate the competitive index and accounted for 87% of the variation in the index.

Mahmoud et al. (1998) found significant correlations between the percentage of fertilized oocytes and sperm motility and also between the percentage of fertilized oocytes and the percentage of spermatozoa with normal morphology. Palmer and Barth (2003) however, found that the percent motile sperm and concentration values were only moderately correlated with each other ($r = 0.22$, $P < 0.01$), by machine and by conventional methods ($r = 0.23$, $P < 0.01$). They further concluded that when compared across machine and conventional methods there were significant, positive correlations for percent motile sperm ($r = 0.82$, $P < 0.001$) and sperm concentration ($r = 0.80$, $P < 0.001$). Budworth et al. (1987), using a microcomputer-photographic system, concluded that there was a correlation between the spermatozoal characteristics, percentage of motile

sperm or spermatozoa velocity, and the competitive fertility index (r = 0.82, $P < 0.05$ and r = 0.83, $P < 0.05$; respectively). Mahmoud et al. (1998) concluded that SMI values correlated better with the percentage of fertilized oocytes than most conventional semen parameters ($r \approx 0.5$, $P < 0.05$ for SMI vs. $r \approx 0.33$ or 0.46, $P < 0.05$).

Ericsson et al. (1993) stated that fluorogenic stains may be useful in identifying an elite population of sperm cells that have a greater chance of completing the fertilization process. However at the conclusion of their study the authors stated that none of the semen quality parameters, flow cytometry or classical measures, were correlated with fertility estimates as defined by ability to complete the fertilization process. Classical measures were defined as post-thaw motilities, intact acrosomes, normal morphology, abnormal heads, vacuoles-craters and spermatozoa with attached cytoplasmic droplets (Ericsson et al., 1993).

*Seminal Parameters*

Objective measurements of sperm quality traits give better predictions of fertility than the subjective measurement of visual estimation (Saacke, 1982). Visual estimation is the most commonly accepted way of evaluating spermatozoa traits but this estimation technique is not highly repeatable or reliable when predicting fertility (Linford et al., 1976; Graham et al., 1980; Moce & Graham, 2008). The 95 % confidence interval is approximately ± 20 percentage units for visual determination of percent motile spermatozoa (Budworth, 1987). Beef Improvement Federation guidelines (2010) state that semen evaluation provides important information relative to a bull's fertility, with sperm viability and morphology among the most important information from the semen evaluation to be used as an indicator of a bull's fertility (Guidelines, 2010). The Beef

Improvement Federation guidelines have been updated through the years as new technologies emerge and have been written by committees, established by the Beef Improvement Federation Board of Directors, to develop recommendations based on scientific research results and industry experience.

An alternative is to use more objective methodologies through use of computer visualization instead of relying on the subjectivity of the human eye. Traits that lend themselves to this method consist of motility, cell velocity, path traveled, concentration, cell size, and cell shape. Motility can be quantified through two different measurements. One is overall sample motility and the other is specific track motility measured for each individual sperm cell.

Velocity is measured, by the computer, three different ways for each cell (linear, curvilinear, and straight line), and is classified as slow, medium or rapid. Average pathway velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL) are the three most commonly used measures of sperm movement (Figure 1). VAP is the smoothed average position of the sperm cell, VSL is the straight-line distance between the beginning and the end of the cell path, and VCL is the total distance traveled by the sperm cell including every point in the cell path all divided by the time elapsed and expressed in micrometers per second. Linearity (LIN), straightness (STR), and wobble (WOB) are all ratios of the velocity parameters (VSL/VCL, VSL/VAP, and VAP/VCL, respectively) used for looking at progressiveness on a relative scale and are expressed as a percentage.

Amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) are both measures of cell oscillation and are based on each specific sperm cell path.

Amplitude of lateral head displacement is the measurement of the maximum value of the distance, in µm, of any point on the cell track from the average path, multiplied by two (Figure 1). BCF measures the frequency with which the cell track crosses the average cell path in either direction and is expressed in Hz (number of video frames per second) (Hamilton Thorne Research, Beverly, MA, USA; Mortimer, 1994; Farrell et al., 1998; Hoflack et al., 2007).

Cell size, cell shape and cell pixels are used as indicators of sperm cells vs. debris, and cell abnormality (Hamilton Thorne Research, Beverly, MA, USA). Budworth et al. (1988) concluded that a size range of 20 to 70 pixels was optimal for discriminating all motile and immotile cells from debris.

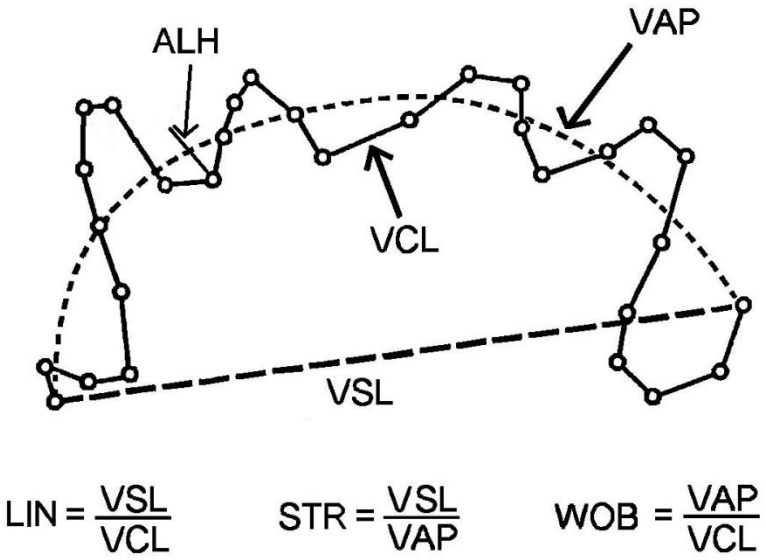


Figure 1. Definitions of cell velocities and motion parameters. Adapted from Hamilton Thorne Research, Beverly, MA, USA

The question posed now is how or if these parameters, analyzed by computer, are related or able to predict fertility. Many studies have been done to analyze seminal

parameters and their relationship to fertility; however, results differ greatly. As early as the 1940's laboratory tests of semen quality were related to fertility (Swanson and Herman, 1944; Branton et al., 1951; Buckner et al., 1954; and Bratton et al., 1956). These four studies defined fertility as the presence of a calf, palpation for the presence of a fetus, or failure of a cow to return to heat within 90 d after insemination (Swanson and Herman, 1944); percent 60- to 90- d non return rate to first service (Branton et al., 1951; Buckner et al., 1954); and the percent of first service cows not returning for reinsemination within 60 to 90 d after the mo in which they were inseminated with semen extended 1:300 in 3.6% citrate-sulfanilamide-yolk (Bratton et al., 1956). Concentration of spermatozoa in the ejaculate, the percent of motile sperm, and sperm velocity were found to be significantly correlated with fertility (Bratton et al., 1956; Budworth et al., 1987). Budworth et al., (1987) calculated the competitive fertility index which is a ranking based on relative fertility and is not a direct estimate of fertility. Beat cross frequency and ALH were found to be not significantly correlated with fertility, as defined as the percentage of cows and heifers that apparently were pregnant 75 d after the insemination for experiment 1 and by calculating the competitive fertility index for experiment 2 (Budworth et al., 1988). In 1985 a study was published by Makarechian and Farid who concluded that none of the semen characteristics they analyzed were able to predict fertility. There is a large range of correlations in literature for sperm motility and fertility, as low as 0.15 to as high as 0.83 (Kjaestad et al., 1993; Bailey et al., 1994; Stålhammar et al., 1994; Januskauskas et al., 2003). Evaluations of track motility, though repeatable and precise, have been found to be only slightly more reliable in predicting fertility of bull sperm than visual estimation (O'Connor et al., 1981; Saacke et al., 1980).

The average percentage of abnormal spermatozoa has not been found to be linearly correlated with fertility (Bratton et al., 1956). However, Saacke (2004) found that sperm with classically abnormal heads or tails are unable to move along the female reproductive tract, and thus are unable to participate in fertilization. Saacke (2004) also suggested that the presence of abnormal sperm in semen may reduce reproductive efficiency due to insufficient numbers of sperm that are able to reach the site of fertilization, the cells inability to fertilize the oocyte, or the inability to sustain the embryo post-fertilization. Kastelic and Thundathil (2008) also found that fertility is decreased with > 30% morphologically abnormal sperm or > 20% head defects. Hoflack et al. (2007) found the percentage of abnormal tails and distal droplets to be negatively correlated with the percentage of motile, progressively motile, and rapid spermatozoa, and positively correlated with the percentage of static spermatozoa.

Mukasa-Mugerwa (1974) conducted his PhD dissertation on the genetics of bovine spermatozoan morphology and reported heritabilities for semen traits in bovine and mice in his literature review from seven different papers. Bovine heritabilities are as follows; semen volume had the greatest range of 0.10 to 0.62, concentration was 0.10 to 0.40, viability was 0.10 to 0.36, motility was 0.13 to 0.23, percent normal was 0.24, percent live was 0.06, percent primary abnormalities were 0.13 to 0.30, and percent secondary abnormalities were 0.05 to 0.23. In mice the author reported heritabilities for head length, midpiece length and head shape of 0.72 ± 0.18, 0.97 ± 0.36, and 0.90 respectively. The author calculated repeatability estimates for fourteen semen parameters:

- Volume,
- Concentration,
- Percent motility,
- Percent normal spermatozoa,
- Percent abnormal spermatozoa,
- Percent head abnormalities,
- Percent midpiece abnormalities,
- Percent abaxial attached midpieces,
- Percent detached heads,
- Percent tail abnormalities,
- Percent proximal droplets,
- Percent distal droplets,
- Percent primary abnormalities, and
- Percent secondary abnormalities.

The range of repeatabilities was from 0 to 94.8% with the average of repeatabilities around 62.5%.

Another possible effect on sperm production would be age of bull greater than one year. Coulter and Kozub (1989) found a decrease in abnormal sperm traits, incidence of secondary sperm defects and percentage of sperm with an abnormal acrosome, as bull's age from 1 year to 2 years but the percentage of progressively motile sperm declined as bulls reached 3 years of age. The authors also found that as bull age increased so did ejaculate volume and sperm concentration. The increase in ejaculate volume and

sperm concentration could be due to the decreased seminal quality, which is best estimated objectively using a calibrated machine.

*Estimated Conception Rates*

Estimated Relative Conception Rate (ERCR) was implemented by the Animal Improvement Programs Laboratory (AIPL) in May of 2006 when AIPL assumed the responsibility of the US evaluation of service sire fertility in dairy cattle. ERCR was developed by Dairy Records Management Systems and North Carolina State University (Raleigh, NC), and evaluations were computed and published by Dairy Records Management Systems. Clay and McDaniel (2001) defined ERCR as an estimate of the difference of a bull, used for AI mating, from the average bull, used for AI mating, of herdmates for the 70d rate of nonreturn. The model the authors utilized for computing ERCR is as follows: NR70 = HYM+ECM+P+DOI+ERCR+Animal+PE+e. Where: NR70 = 70-day nonreturn status of cow(1=no return, 0=return), HYM = herd-year-month of breeding, ECM = average of daily, early-lactation, energy-corrected milk, P = parity, DOI = days open at first mating, ERCR = for rate of return in 70 d, expressed as the difference of one AI mating bull from the average AI mating bull of herdmates (uncorrelated random effect), Animal = random effect of animal including additive relationships, PE = permanent cow effect (uncorrelated random effect), and e = residual. Kuhn and Hutchison (2008) noted that the 70 d non-return rate used in calculating ERCR is for first service only and does not include subsequent matings.

Clay and McDaniel (2001) concluded that bull fertility was predictable and repeatable ($r^2 = 0.54$) based on ERCR as long as the number of matings was adequate.

The authors defined adequate as ≥ 300 matings, when the number of matings decreased below 300 the authors found the beta solutions for their equation were not significant.

Sire conception rate (SCR), which is the current measurement of fertility, is defined similarly to ERCR but instead of using the 70 d nonreturn rate, actual cow conception rate is used, validated by pregnancy check or a resulting calving date (USDA, 2010). The change was made after AIPL took over the fertility evaluation (Norman et al., 2008). There are far stricter requirements to being included in an SCR analysis but more breeds are currently analyzed than were in the initial ERCR analysis. Table 3 highlights these requirements and compares ERCR to SCR.

Table 3. Comparison of ERCR and SCR[1,a]

| Category | ERCR | SCR |
|---|---|---|
| Trait evaluated | First service 70 d nonreturn rate | Conception Rate |
| Breeds evaluated | Holstein, Jersey | Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, Milking Shorthorn |
| Lactation numbers included | 1-6, with >6 = 6 | 1-5 |
| Service numbers included | 1 | 1-7 |
| Bulls included | AI, < 12 yr old | AI, not active AI, < 13 yr old |
| Minimum number of matings | ≥ 300 first services | ≥ 300 services in the last 4 yr and ≥ 100 in the last yr for Holsteins; somewhat fewer services for other breeds |
| Minimum number of herds | None | 10 for Holsteins and Jerseys, somewhat fewer for other breeds |
| Fertility expression | Deviation from mean (nearest 1%) | Deviation from mean (nearest 0.1%) |
| Base assigned | Mean value forced to 0 | Mean value forced to 0 |

[1]ERCR = estimated relative conception rate, SCR = sire conception rate
[a]Adapted from Norman et al., 2008

Kuhn et al. (2008) assessed the use of a combination of nuisance variables to adequately and accurately model conception rate (CR). The nuisance variables assessed

were; 1) management group, defined as herd, year, season, parity, and registry status (HYSPR) 2) milk yield 3) cow age 4) days in milk at breeding 5) lactation number 6) service number 7) an interval between breedings variable (to account for lower CR following short cycles) and 8) cow effects, both genetic and permanent environmental. The final model chosen used HYSPR, year-state-month (in combination with HYSPR accounts for climate effects), lactation, service number, milk yield, cow age at breeding, a variable to account for the effect of short intervals between breedings, and the cow effect, partitioned as permanent environment and breeding value. Animal Improvement Programs Laboratory adapted a model with all eight of the previous mentioned nuisance variables partitioned out, and included a service sire random (SSR) effect so as to better estimate true bull fertility. These eight variables were chosen to be included because of the previous research done to approve them for future CR analyses (Kuhn et al., 2008). Norman et al. (2008) listed 5 factors that are associated with the SSR variable; 1) inbreeding of the bull 2) inbreeding of the embryo from the mating 3) age of the bull 4) AI organization combined with the year of the mating and 5) effect of the bull itself. This is the result of research done by Kuhn and Hutchison (2008) who determined that a diagonal variance-covariance matrix was used for the SSR term because the heritability of diary bull fertility with the use of AI was assumed to be zero. The authors also concluded that completely removing sire effect from the prediction of the service sire fertility drastically reduces the accuracy of the evaluation performed.

Fertility in the dairy industry, and any livestock industry, is essential to continuous production of consumables and ultimately to the livelihood of animal producers. If fertility could be estimated at a very early age it would save many producers

the cost of growing and maintaining bulls that are sub- or in-fertile. Scrotal circumference is very important in predicting bull fertility, as are breeding soundness exams, but to take it to the next step and increase our ability to predict fertility, seminal characteristics should be included. This would not only get rid of unsatisfactory potential breeders but help to distinguish between bulls that are considered 'satisfactory potential breeders' in terms of average semen or 'optimum' semen. While this is not currently something that is analyzed, the ability to look at the CASA parameters of a bull and fit a model to most effectively predict fertility would allow producers the option of marketing their bulls as a more satisfactory breeder than others.

# MATERIALS AND METHODS

*Data Description*

Cryo-preserved semen from 120 Holstein bulls was obtained from three semen companies, which were randomly coded 1:3; Semen Company 1(SC1), Semen Company 2 (SC2) and Semen Company 3 (SC3); to decrease bias, through USDA-ARS, Fort Collins, CO. The number of bull samples originating from each semen company is as follows: Semen Company 1 (SC1) - 32, Semen Company 2 (SC2) - 71, and Semen Company 3 (SC3) – 17. Bulls were born over a wide range of years, 1985-2003, and were un-evenly distributed among the years. Computer assisted semen analysis (CASA) (HTM-IVOS, Version 10.8, Hamilton Thorne Research, Beverly, MA, USA) was used to assess seminal characteristics.

The CASA was parameterized as follows: frames acquired – 100, frame rate – 60 Hz, minimum contrast – 70, minimum cell size (SIZE) – 8 pixels, minimum static contrast – 30, straightness threshold – 80%, average pathway velocity (VAP) cutoff – 25 µm/s, straight line velocity (VSL) cutoff – 20 µm/s, cell intensity – 80, static elongation – 11 to 80, magnification – 1.89. 25 µL of post-thaw semen was diluted into 50-100 µL of Tris (formulated for bull semen), and 5 µL of this diluted semen was loaded into a pre-warmed dual chamber slide, and then loaded into the CASA for analysis. Of the 120 bulls utilized, 20 had been previously analyzed for other studies. The CASA data of these 20 bulls were used instead of running another CASA to preserve the limited availability of semen straws. The settings on the CASA for these 20 bulls were the same as the 100

analyzed for this study. The number of sperm cells counted for each bull ranged from 505 to1366 individual cells, depending on the study the CASA was performed for.

The CASA provided two files with results for each of the bulls, one was a summary file (DBS) in which each of the bulls had overall means for each of the parameters and the second (DBT) was a file that had every live cell tracked and the parameters for each cell. The seminal parameters were similar across the two files and consist of VAP, VSL, curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), elongation, SIZE, size in pixels, and intensity. The DBS file stores other variables such as total concentration, percent alive, percent motile and percent progressively motile as well.

For this study the primary CASA outcomes of interest were percent motility, percent progressive, VAP, VSL, VCL, ALH, BCF, STR, LIN and SIZE.

Sperm SIZE was limited to a range of 5.36 to 28.7μm based upon previous literature estimates (Budworth et. al, 1988; Farrell et. al, 1998; Hoflack et.al, 2007); this range included 3 standard deviations from the mean of this study. The average pedigree relationship between bulls in this study was 12.16%, compared to active AI Holstein bulls of 11.5% (Weigel and Lin, 2002). The average relationship between bulls by semen company (1:3) are 12.41%, 12.1% and 13.47%, respectively. The average pedigree inbreeding of the bulls in this study was 5.62% with a minimum of 1.94% and a maximum of 15.37%. The average SCR for the bulls in this study was -1.09 ± 2.58 with the average SCR for active AI sires being 0.799 ± 1.67; only 46.71% of active AI sires have a SCR value.

*Statistical Analysis*

Data were initially analyzed using a statistical software program (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA). A single trait mixed model was fit to analyze each of the seminal parameters; average pathway velocity (VAP), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF); and their transformed variables as needed, the log base 10 of VAP (lVAP) and the square root of ALH (ALH1) (these two transformations were chosen based on preliminary distributional analyses). For each model the fixed effects were the semen company, SIZE, with the random effect being animal nested within semen company. SIZE was fit two ways depending on the data. If the dependent variable was non-normal and untransformed, the cubic effect of SIZE was fit. If the dependent variable was normal or transformed for normality then the inverse effect of SIZE was fit. This was chosen based on initial data exploration, looking at graphs of SIZE vs. each dependent variable.

While some data exploration was conducted on VCL and VSL, VAP was chosen out of the three velocity parameters for statistical analysis because it was the average path the sperm cell took and the three velocity parameters were all highly correlated ($P \leq 0.001$) as shown in Table 4. Secondly, VAP was chosen over VSL because VSL is used mainly by the CASA program to differentiate between "slow motile" and "static" cells.

Table 4. Pearson correlation coefficients of velocity parameters[1].

| Phenotype[1] | VSL | VCL |
|---|---|---|
| VAP (µm/s) | 0.871 | 0.845 |
| VSL (µm/s) | | 0.659 |

[1]Velocity parameters: VAP = average pathway velocity, VSL = straight line velocity, VCL = curvilinear velocity

SIZE and elongation were analyzed as predictors to see if they truly had an effect on the sperm's ability to be motile and progressive. Because data were pulled from a

previous analysis, the effect of study was included in the model and tested as a significant effect, but was not included in the final model. The difference between semen companies was analyzed for each seminal parameter using the 'lsmeans/pdiff' option in the mixed procedure of SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA). The model used to estimate the seminal parameters was:

$$Y = SC + SIZE + Animal(SC) + e$$

where:

Y = CASA variable (VAP, lVAP, ALH, ALH1, BCF)
SC = Company semen was obtained from
SIZE= the effect of cell SIZE (cubic or inverse)
Animal(SC) = random effect of animal nested within semen company
e = residual.

After analyzing the individual cell data for the CASA variables, the "Mixed" procedure of SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA) was used to estimate the effect of CASA variables on sire conception rate. Due to the nature of only one SCR value per bull, the data for the CASA variables were averaged to allow for convergence. The model used to estimate fertility was:

$$SCR = SC + SIZE + CASA + Animal(SC) + e$$

where:

SCR = Sire Conception Rate
SC = Company semen was obtained from
SIZE= the effect of cell SIZE
CASA = CASA variable (lVAP, ALH1, BCF)
Animal(SC) = random effect of animal nested within semen company
e = residual.

The ultimate goal of the preliminary analysis was to determine the appropriate fixed effects for inclusion in the model estimating heritability. The next step was then to estimate those heritabilities.

A single trait analysis was then performed looking at each of five seminal parameters, and the fertility estimate; VAP, ALH, BCF, SIZE, percent motile and SCR. This analysis was performed using ASReml (Gilmour et al., 2006), ASReml uses the average information procedure of REML and sparse stored matrices. Each model was fit with an additive direct, permanent environment (not included in SCR analysis due to only one SCR value per bull), and residual effect which were used to calculate phenotypic, genetic and residual variances for each parameter. After residual variances were estimated heritabilities and repeatabilites of each of the parameters were estimated. Pedigree data obtained included animal, sire, and dam and included 18 generations. The confounding of animal and semen company is overcome by including this relationship matrix in the equations.

A multi trait analysis was run to estimate the genetic correlation between SCR and CASA values. The random animal model used to estimate genetic correlations was as follows:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where:

$y_1$ = Sire conception rate
$y_2$ = CASA variable (VAP, ALH, BCF, SIZE, % motile)
$X_i$ = known incidence matrix for fixed effects
$Z_i$ = known incidence matrix for additive genetic effects
$e_i$ = residual effects.

# RESULTS AND DISCUSSION

*Raw Data*

The mean, standard deviation, and range values for CASA seminal parameters are presented in Table 5. Percent motile and percent progressive sperm cells for this study were equal to or greater than other CASA utilizing cryo-preserved semen (Budworth et. al, 1987; Budworth et. al, 1988); but were lower than the CASA results from fresh semen studies by 10 to 20 % (Farrell et. al, 1998; Hoflack et. al, 2007). When looking at the velocity parameters; average pathway velocity (VAP), curvilinear velocity (VCL), and straight line velocity (VSL), the results from this study were approximately equal to studies that analyzed cryo-preserved semen (Budworth et. al, 1987; Budworth et. al, 1988) and were lower than studies that analyzed fresh semen by 30 – 70 μm/s (Farrell et. al, 1998; Hoflack et. al, 2007). This is to be expected due to death loss of sperm cells during the freezing process. Budworth et. al used only nine beef bulls (1987) ten dairy bulls (1988) for their analyses so they had 10% of the bulls that this analysis used. Amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) for this study (8.45 and 30.99, respectively) were both greater than other cryo-preserved and fresh semen studies; 3.2 and 15.9 (Budworth et al., 1988), 5 and 15 (Farrell et al., 1998), except one study had a greater BCF, 4.83 and 37.4 (Hoflack et al., 2007). Straightness (STR, VSL/VAP) for this study was lower than estimates previously determined (Farrell et al., 1998, Hoflack et al., 2007); however, the cited studies utilized fresh semen instead of cryo-preserved semen.

Table 5. Number of (N), mean, SD, coefficient of variation and range for all CASA outcomes.

| Phenotype[1] | N | Mean ± SD | CV[2] | Range |
|---|---|---|---|---|
| Motility (%) | 70586 | 60.93 ± 10.09 | 16.72 | (29 – 82) |
| Progressive (%) | 70586 | 32.46 ± 10.06 | 31.00 | (10 – 55) |
| VAP (μm/s) | 70586 | 111.37 ± 40.97 | 36.78 | (25 – 425.1) |
| VSL (μm/s) | 70586 | 90.69 ± 40.60 | 44.77 | (20 – 421.8) |
| VCL (μm/s) | 70586 | 193.93 ± 71.50 | 36.87 | (25.3 – 590) |
| ALH (μm) | 70586 | 8.45 ± 3.5 | 41.45 | (0.2 – 26.5) |
| BCF (Hz) | 57450 | 30.99 ± 9.27 | 29.90 | (4.8 – 59.4) |
| STR (VSL/VAP) | 70586 | 80.74 ± 17.11 | 21.19 | (10 – 100) |
| LIN (VSL/VCL) | 70586 | 48.24 ± 16.15 | 33.47 | (6 – 100) |
| SIZE ($\mu m^2$) | 70586 | 8.38 ± 2.88 | 34.34 | (5.4 – 28.7) |

[1]VAP = average pathway velocity, VSL = straight line velocity, VCL = curvilinear velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency, STR = straightness, LIN = linearity
[2]CV = coefficient of variation

Fresh semen should have sperm with less damage which would account for greater straightness. Mukhopadhyay et al. (2011) found that sperm DNA fragmentation significantly ($P \leq 0.01$) increased due to cryopreservation and thawing of sperm cells as compared to fresh semen.

Linearity (LIN, VSL/VCL) in this study was 48.24% which was comparable to the average of results found in previous studies (39.34%; Farrell et al., 1998, Budworth et al., 1988). Computer assisted semen analysis using fresh semen calculated LIN at 82-88% (Farrell et al., 1998) versus CASA using cryo-preserved semen found results for LIN at 6% (Budworth et al., 1988), this difference could be attributed to damage from freezing. A reason STR and LIN would be different from literature estimates would be because these are functions of the velocity parameters and if great differences exist in the velocities of semen analyzed, it would most likely be seen in these ratios. These ratio traits were no included in the final models due to the problem of increased selection pressure on ratio traits. Gunsett (1984) found that selecting directly for the ratio trait changed the selection pressure of the components of that ratio in a nonlinear fashion.

SIZE for this study was within literature estimates (Budworth et al., 1988; Farrell et al., 1998; Hoflack et.al., 2007).

*Untransformed Data*

The untransformed data set was utilized for prediction models. In the initial data exploration, graphs revealed a trend for SCR to decrease slightly as velocities (VAP, VSL and VCL) increased (Figures 2, 3 and 4). This trend was also seen when looking at the relationship between SCR and ALH or BCF (Figures 5 and 6). When SIZE was plotted against the velocity parameters SIZE had a cubic relationship on all three velocities (Figure 7). The biggest difference was seen in VCL, it appears that the size of the cell could have an effect on the cells ability to swim in a straight line, with an ideal SIZE between 7 and 8 µm as evidenced by the lowest curvilinear velocity in Figure 7. This cubic relationship was also seen when looking at SIZE and ALH or BCF (Figures 8 and 9). Looking at the data graphed without being transformed provided insight into using the cubic effect of size (SIZE3) as an additive effect in SAS models.

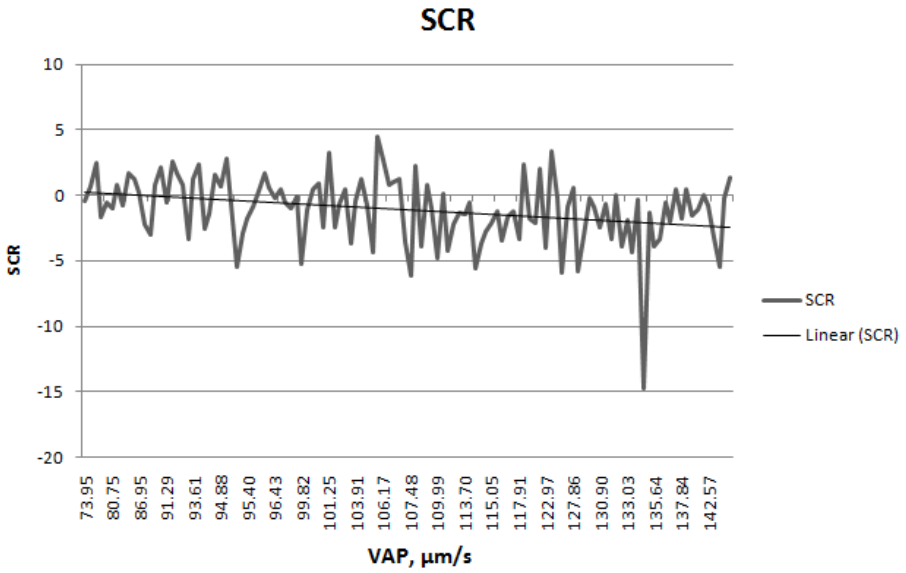


Figure 2. Linear regression of sire conception rate (SCR) on average pathway velocity (VAP).

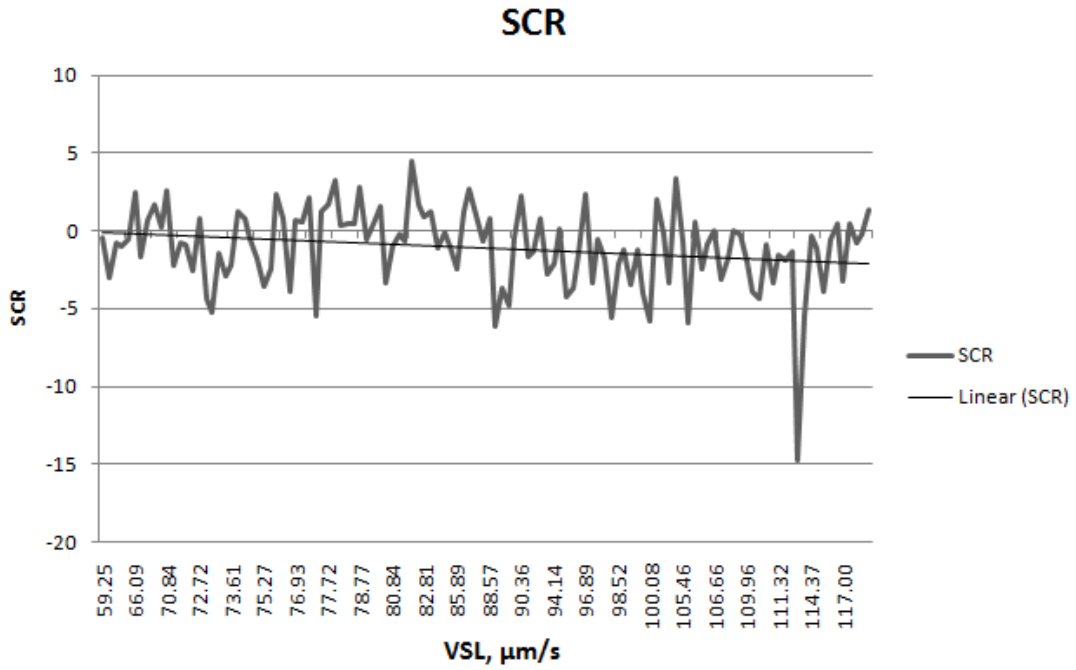


Figure 3. Linear regression of sire conception rate (SCR) on average straightline velocity (VSL).

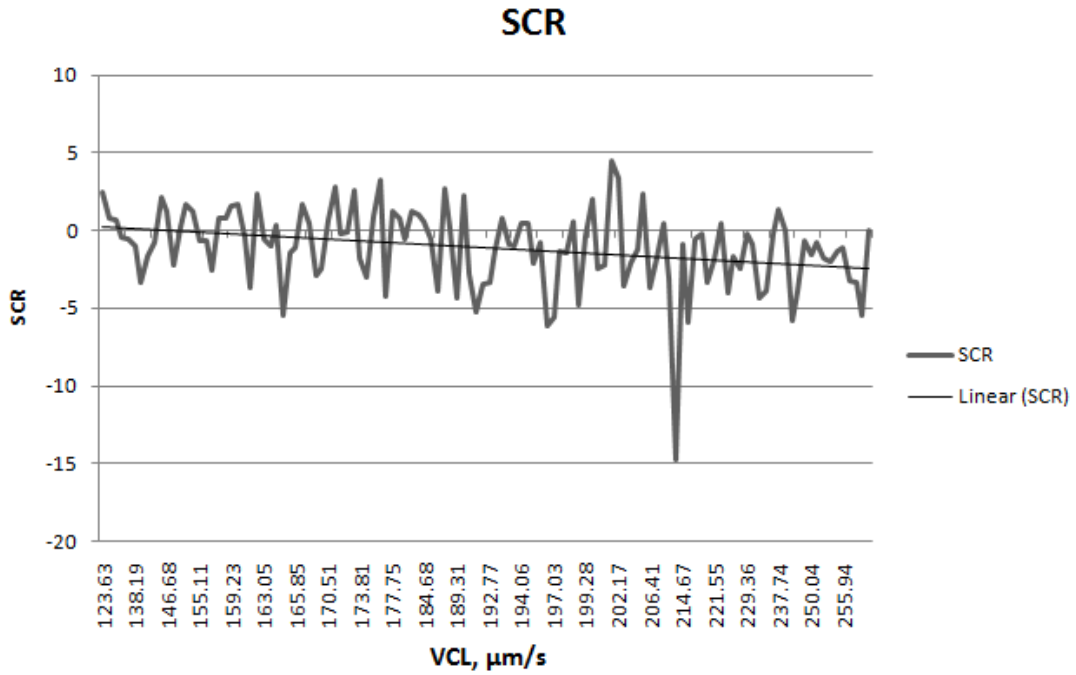


Figure 4. Linear regression of sire conception rate (SCR) on average curvilinear velocity (VCL).

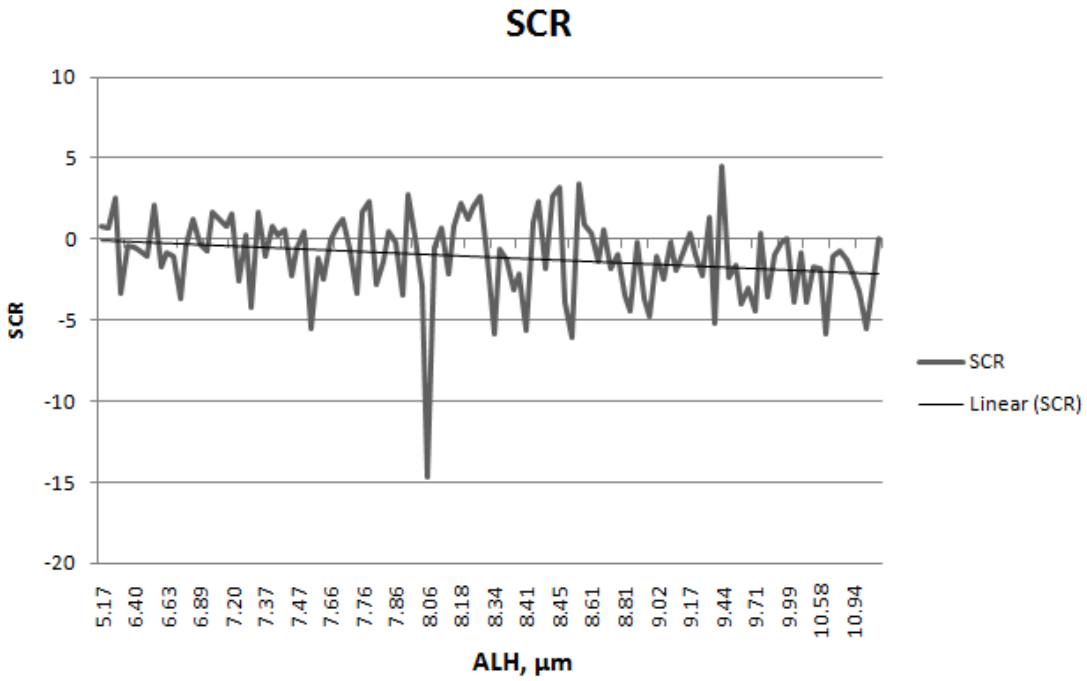


Figure 5. Linear regression of sire conception rate (SCR) on average amplitude of lateral head displacement (ALH).

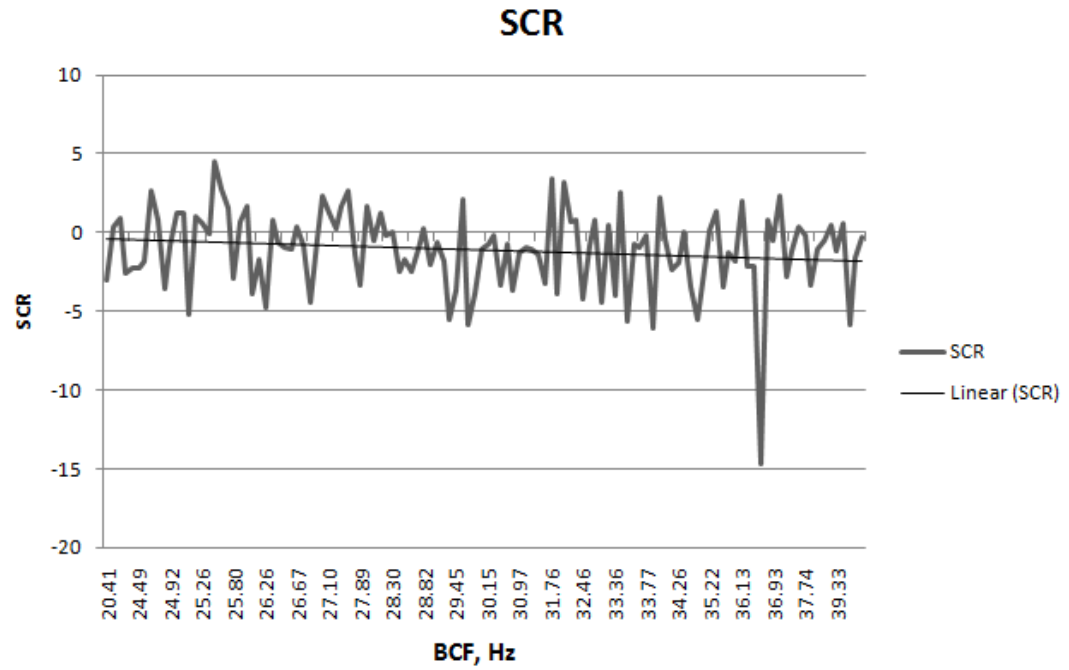


Figure 6. Linear regression of sire conception rate (SCR) on average beat cross frequency (BCF).

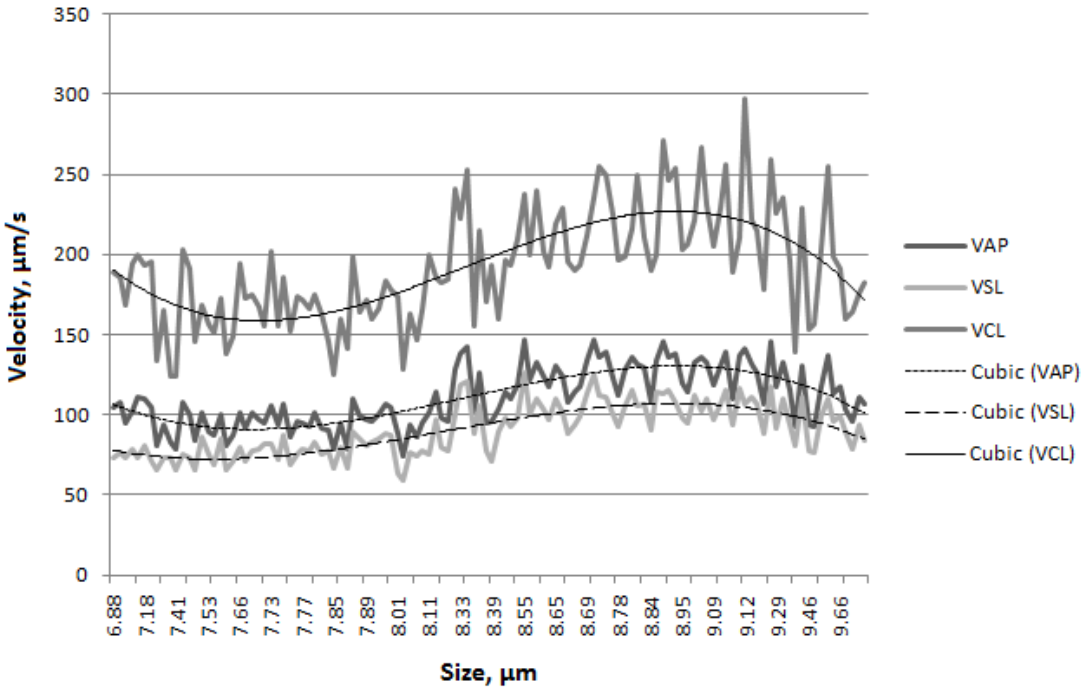


Figure 7. Relationship between average cell size and average pathway velocity (VAP), average straightline velocity (VSL), and average curvilinear velocity (VCL).

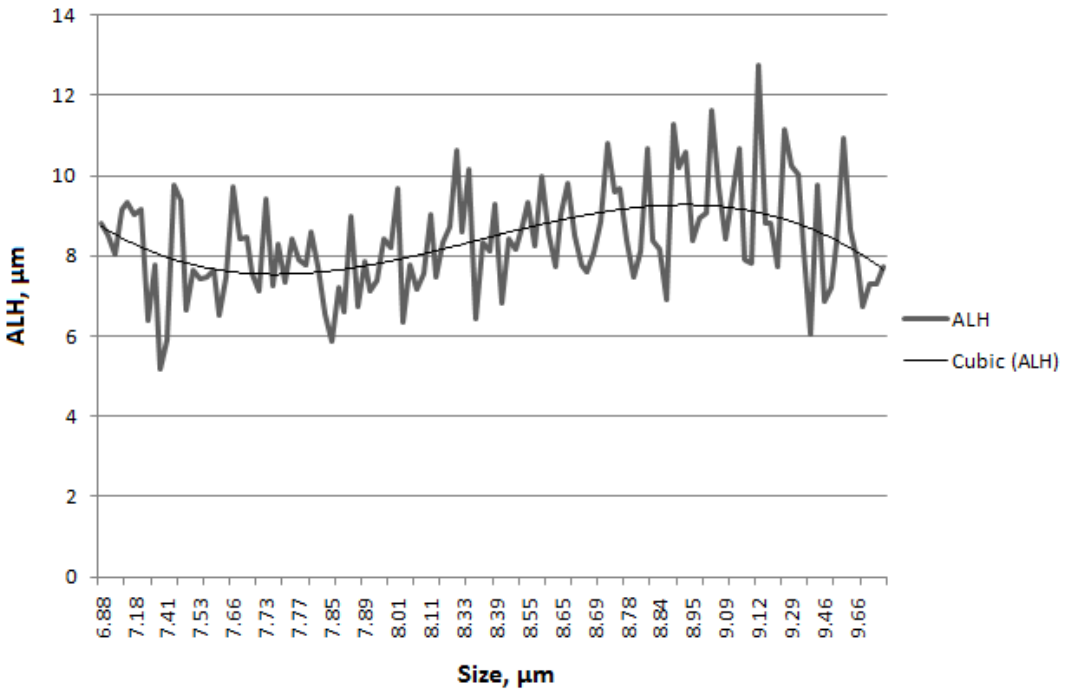


Figure 8. Relationship between average cell size and average amplitude of lateral head displacement (ALH).

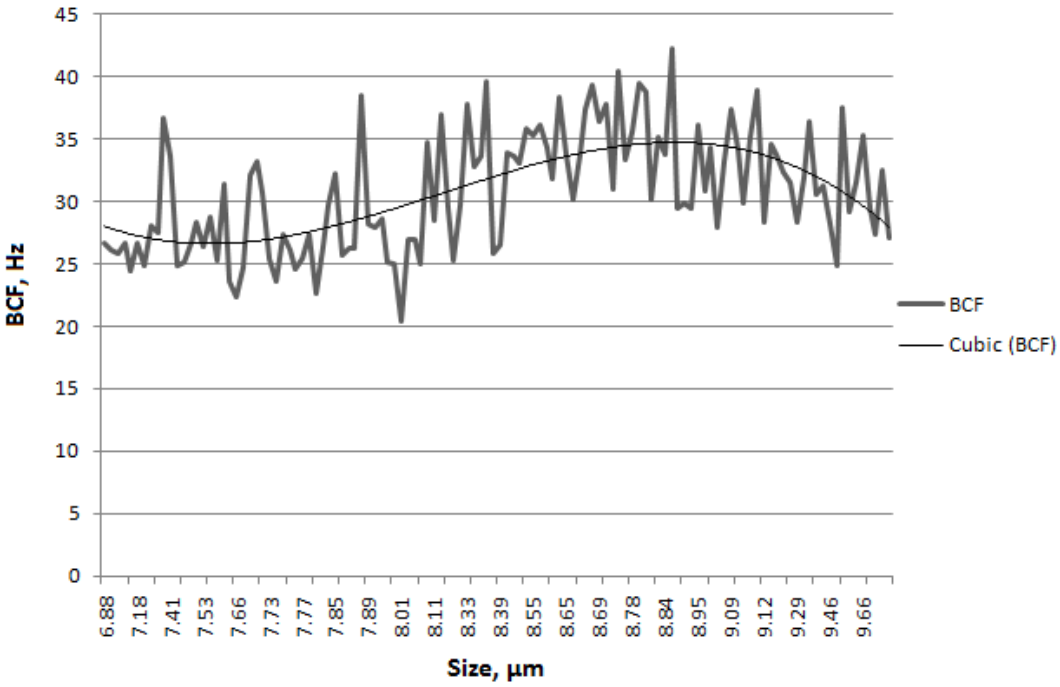


Figure 9. Relationship between average cell size and average beat cross frequency (BCF).

Initial model testing in SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA) revealed that cell elongation had a significant effect ($P < 0.01$) on sperm parameters, when fit as an additive effect to SIZE, but alone elongation was not significant. For this reason, elongation was left out of the model and SIZE was chosen as a fixed effect for mixed model analysis. Elongation and SIZE were, however, only lowly correlated ($r = 0.061$, $P < 0.001$). As stated previously, due to the evidence of cubic effect on velocity parameters, SIZE3 was included in the model as a fixed effect. Because data were utilized from semen samples from previous studies, study source was tested as a fixed effect in the mixed models. Mean SIZE for each bull was also tested to see if there were differences in distributions using the Student Newman-Keuls (SNK) and Ryan-Einot-Gabriel-Welsch Q (REGWQ) means options in the glm procedure of SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA). While 119 of the bulls' means

overlapped in succession, one bull was found to have an average SIZE that was significantly different ($P < 0.01$) from the other 119 bulls using both tests.

When semen company was fit in the models it was found to be significant ($P \leq 0.05$). For this reason this study also looked at the differences of least squares means [$H_0$: $LSMean(SC_a)=LSMean(SC_b)$] between the semen companies for each untransformed seminal parameter, this data is presented in Table 6. It is interesting to note that only one pair of companies was found to be significantly different ($P \leq 0.001$) across all the seminal parameters, SC1 and SC2. Significant differences between the other semen companies were detected for SC1 and SC3 for the parameters ALH ($P \leq 0.05$) and BCF ($P \leq 0.001$); when looking at SC2 and SC3 significant differences were detected between VAP ($P \leq 0.001$) and ALH ($P \leq 0.001$). For this reason semen company was included in the genetic model to reduce error variation.

Table 6. Differences of least squares means, standard errors and P-values[1] between semen companies[2] for each untransformed seminal parameter and SCR.[3]

| Semen Companies | | VAP | ALH | BCF | SCR |
|---|---|---|---|---|---|
| SC1 | SC2 | -24.66 ± 2.85 | -1.19 ± 0.232 | -6.142 ± 0.845 | 2.1863 ± 0.447 |
| | | <0.001 | <0.001 | <0.001 | <0.001 |
| SC1 | SC3 | 3.656 ± 4.02 | 0.842 ± 0.327 | -5.623 ± 1.19 | 0.06 ± 0.62 |
| | | 0.365 | 0.011 | <0.001 | 0.923 |
| SC2 | SC3 | 28.32 ± 3.61 | 2.031 ± 0.294 | 0.519 ± 1.07 | -2.126 ± 0.591 |
| | | <0.001 | <0.001 | 0.628 | <0.001 |

[1]Estimate is followed by its standard error, p-values are listed below estimates.
[2]Semen companies: SC1 = Semen Company 1, SC2 = Semen Company 2, SC3 = Semen Company 3.
[3]Seminal parameters: VAP = average pathway velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency, SCR = sire conception rate.

Phenotypic, genetic, and residual variances of the seminal parameters are presented in Table 7 with their standard errors. The heritabilities and repeatabilities of the seminal parameters are presented in Table 8 and ranged from 0.009 to 0.785. Repeatability is not calculated for percent motile and SCR due to there being only one

observation per animal. Percent motile sperm had the lowest residual variance compared to other seminal parameters. Percent motile sperm and SCR were estimated to be highly heritable (0.785 ± 0.302 and 0.629 ± 0.305, respectively) with the other seminal parameters ranging from 0.009 to 0.088. Brun et al. (2010) utilized rabbit semen for CASA and genetic analysis and found heritability of VAP and LIN to be low (0.14 ± 0.03 and 0.05 ± 0.03, respectively) with percent motile sperm having the highest heritability of 0.18 ± 0.04. While Brun et al. (2010) also found percent motile to be highly heritable their estimate is much lower than this study's estimate of heritability. This could be attributed to the difference in species and this study utilizing proven bulls who had multiple ejaculates taken over time. Brun et al. (2010) still concluded that percent motile sperm appeared to be a good criterion to aid in the selection of semen quality.

Table 7. Estimates of genetic, phenotypic and residual variances of untransformed seminal parameters with their standard errors.

| Seminal Parameters[1] | Variances | | |
|---|---|---|---|
| | Genetic | Phenotypic | Residual |
| VAP | 135.3 ± 59.86 | 1536 ± 27.60 | 1352 ± 7.20 |
| ALH | 0.346 ± 0.373 | 11.68 ± 0.173 | 10.51 ± 0.056 |
| BCF | 5.427 ± 5.659 | 78.81 ± 2.301 | 62.66 ± 0.367 |
| SIZE ($\mu m^2$) | 0.075 ± 0.141 | 8.218 ± 0.079 | 7.726 ± 0.041 |
| Motile (%) | 82.76 ± 38.89 | 104.4 ± 15.58 | 21.62 ± 30.07 |

[1]VAP = average pathway velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency.

Table 8. Estimates of heritability and repeatability of untransformed seminal parameters with their standard errors.

| Seminal Parameters[1] | Heritability | Repeatability |
|---|---|---|
| VAP | 0.088 ± 0.038 | 0.119 ± 0.015 |
| ALH | 0.027 ± 0.032 | 0.101 ± 0.013 |
| BCF | 0.064 ± 0.069 | 0.203 ± 0.023 |
| SIZE ($\mu m^2$) | 0.009 ± 0.017 | 0.06 ± 0.008 |
| Motile (%) | 0.785 ± 0.302 | - |
| SCR | 0.692 ± 0.305 | - |

[1]VAP = average pathway velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency, SCR = sire conception rate.

Phenotypic, genetic, and residual correlations between seminal parameters and SCR are presented in Table 9 with their standard errors. Genetic correlations of VAP, ALH and SIZE with SCR were low (0.05, 0.006, and 0.04 respectively). Phenotypic correlations were low for VAP (-0.071), ALH (0.0647) and BCF (0.0648) but varied greatly. The genetic correlation between BCF and SCR was moderate (0.1413); the phenotypic correlation between SIZE and SCR was also moderate (0.1216). Once again percent motile had the highest correlation with SCR (0.302). The standard errors for all the correlations were moderate to high. Budworth et al. (1988) estimated correlations between CASA values and two different fertility measures, 1) 75-d non-return rate and 2) their calculated competitive fertility index which was calculated by thawing cryopreserved semen at 35 C and mixing equal numbers of spermatozoa from two bulls, when 785 beef cows were inseminated with the mixtures and the sire of each calf was determined by phenotypic markers and bloodtyping. The competitive fertility index ranked the bulls based on relative fertility or competitive fertility. In this study percent motile spermatozoa and the competitive fertility index were highly correlated (0.86) and the 75-d non-return rate was found to be moderately correlated with percent motile sperm (0.34). Budworth et al. (1988) also found that BCF and ALH were not significantly ($P < 0.05$) correlated with either measure of fertility.

As another measure of dispersion, a coefficient of variation (CV) was calculated for all seminal traits measured and is shown in Table 5. Average pathway velocity and VCL were very close at 36.78% and 36.87%, respectively. Straightline velocity had the highest CV at 44.77% followed closely by ALH at 41.45%. Percent motile had the lowest CV at 16.72%, the next lowest calculated was STR at 21.29%.

Table 9. Estimates of genetic, phenotypic and residual correlations between SCR and untransformed seminal parameters with their standard errors.

| | Correlations | | |
|---|---|---|---|
| Seminal Parameters[1] | Genetic | Phenotypic | Residual |
| VAP | 0.054 ± 0.162 | -0.071 ± 0.088 | -0.112 ± 0.114 |
| ALH | 0.006 ± 0.164 | 0.065 ± 0.092 | 0.085 ± 0.115 |
| BCF | 0.141 ± 0.163 | 0.065 ± 0.106 | 0.032 ± 0.132 |
| SIZE ($\mu m^2$) | 0.041 ± 0.152 | 0.122 ± 0.108 | 0.158 ± 0.155 |
| Motile (%) | 0.302 ± 0.293 | 0.032 ± 0.101 | -1.398 ± 0.278 |

[1]Seminal parameters: VAP = average pathway velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency.

Percent progressively motile sperm (31%), BCF (29.9%), LIN (33.47%) and SIZE (34.34%) all had CV's between 29.90% and 34.34% as shown in Table 5. The great variation in CV illustrates the differences between the physiological processes of the bulls utilized in this study.

*Transformed Data*

Data that did not show a normal distribution in preliminary analysis were transformed to meet normality requirements. Average path velocity was transformed using log base 10 (lVAP), ALH was transformed using the square root of the data (ALH1), and SIZE was transformed using the inverse of the cell SIZE data (iSIZE). These were chosen by using the univariate procedure in SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA) and using the tests for normality. In addition, looking at the normal probability plot gave a good visual representation of normality. Initial model testing in SAS (SAS Version 9.2 for Windows, SAS Institute, Cary, NC, USA) revealed iSIZE to have a significant effect ($P < 0.01$) on seminal parameters when fit as a fixed effect in the mixed model analysis.

The transformed data was also analyzed for differences of least squares means [$H_0$: $LSMean(SC_a)=LSMean(SC_b)$] between the semen companies (Table 10). Significant differences ($P < 0.001$) were observed between SC1 and SC2, and between SC2 and SC3

for both lVAP and ALH1. Only small differences between SC1 and SC3 in lVAP were found, but it was not significant ($P = 0.23$) and when looking at ALH1 the difference was significant at $P < 0.01$.

Phenotypic, genetic and residual variances of the transformed seminal parameters are presented in Table 11 with their standard errors. Estimates of heritability and repeatability are presented in Table 12. The heritabilities were low, which is to be expected with the data transformed as it was. The standard errors for heritabilities and variances are as large as or larger than the estimates.

Table 10. Differences of least squares means, standard errors and P-values[1] between semen companies[2] for each transformed seminal parameter.[3]

| Semen Companies | | lVAP | ALH1 |
|---|---|---|---|
| SC1 | SC2 | -0.096 ± 0.012 | -0.200 ± 0.041 |
| | | ≤ 0.001 | ≤ 0.001 |
| SC1 | SC3 | 0.019 ± 0.016 | 0.157 ± 0.057 |
| | | 0.237 | ≤ 0.01 |
| SC2 | SC3 | 0.115 ± 0.015 | 0.357 ± 0.051 |
| | | ≤ 0.001 | ≤ 0.001 |

[1]Estimate is followed by its standard error, p-values are listed below estimates.
[2]Semen companies: SC1 = Semen Company 1, SC2 = Semen Company 2, SC3 = Semen Company 3.
[3]Seminal parameters = lVAP = $\log_{10}$(average pathway velocity), ALH1 = √amplitude of lateral head displacement.

Table 11. Estimates of genetic, phenotypic and residual variances of transformed seminal parameters with their standard errors.

| Seminal Parameters[1] | Variances | | |
|---|---|---|---|
| | Genetic | Phenotypic | Residual |
| lVAP | 0.002 ± 0.001 | 0.031 ± 0.0005 | 0.028 ± 0.0001 |
| ALH1 | 0.009 ± 0.012 | 0.394 ± 0.005 | 0.358 ± 0.002 |
| iSIZE ($\mu m^2$) | 0.00002 ± 0.00003 | 0.0009 ± 0.00001 | 0.0007 ± 0.000004 |

[1]Seminal parameters = lVAP = $\log_{10}$(average pathway velocity), ALH1 = √amplitude of lateral head displacement, iSIZE = 1/SIZE.

Table 12. Estimates of heritability and repeatability of transformed seminal parameters with their standard errors.

| Seminal Parameters[1] | Heritability | Repeatability |
|---|---|---|
| lVAP | 0.074 ± 0.033 | 0.10 ± 0.013 |
| ALH1 | 0.023 ± 0.029 | 0.093 ± 0.012 |
| iSIZE ($\mu m^2$) | 0.025 ± 0.031 | 0.105 ± 0.013 |

[1]Seminal parameters = lVAP = $\log_{10}$(average pathway velocity), ALH1 = √amplitude of lateral head displacement, iSIZE = 1/SIZE.

Phenotypic, genetic and residual correlations between transformed seminal parameters and SCR are presented in Table 13 with their standard errors. Correlations were very similar in nature to the untransformed data. All correlations were low for lVAP and AHL1.

Table 13. Estimates of genetic, phenotypic and residual correlations between SCR and transformed seminal parameters with their standard errors.[1]

| | lVAP | ALH1 |
|---|---|---|
| Genetic correlation | 0.0524 ± 0.1628 | 0.0203 ± 0.1679 |
| Phenotypic correlation | -0.0326 ± 0.0912 | 0.0529 ± 0.0940 |
| Residual correlation | -0.0576 ± 0.1156 | 0.0646 ± 0.1157 |

[1]Seminal parameters = lVAP = $\log_{10}$(average pathway velocity), ALH1 = √amplitude of lateral head displacement.

# CONCLUSIONS AND IMPLICATIONS

While considerable variance was evident between the different semen parameters evaluated, much of this could be attributed to either the physiological process of sperm production or other environmental factors resulting in low heritability. Foote (1970) stated that much of the variation in fertility is not due to genetics. The low heritability of the seminal parameters makes these traits poor candidates for genetic evaluation. While overall variance was high, the CV's calculated were lower than expected on the current data set. Semen production is a physiological process with many steps which leaves many places for things to go wrong but it is important to the industry so limiting errors, if possible, in each of these steps better allow for CASA use in the future.

Significant differences in the least squares means between the semen companies utilized in this study suggest that seminal quality, as measured by CASA parameters, and fertility, as measured by SCR, are different between the companies. This could be attributed to different bull selection methods, different budgets or even different seminal collection methods. The methods semen companies use to dilute semen, extend semen and the procedures for freezing semen are proprietary and would be difficult to find out to truly attribute differences between the semen companies. Each semen company may have different production goals for the bulls they select such as, milking in dairy bulls, carcass production for progeny headed to the feedlot, and days to puberty for replacement heifers in a cow-calf unit. However, the bulls utilized in this study were all dairy bulls so if differences exist in production goals they would be the difference between breeding for

a replacement heifer (sexed versus non-sexed semen), increased milking ability, increased calving rate, or increased stayability. This study also found that average relationship within semen company was approximately equal to or slightly greater than the average of all the bulls. This could show a bias for semen companies to select for bulls along specific genetic lines.

While basic CASA values exhibit low heritability in this study, the percent motile sperm was found to be highly heritable. This supports the use of a sperm motility parameter in breeding soundness exams, and as a criterion for genetic selection. SIZE had a cubic effect on VAP, VCL, VSL, ALH and BCF, and this could lead to genetic selection for an 'optimal' cell size; however, the heritability for these outcomes were low. It is important to note that this study utilized an 18 generation pedigree to analyze the genetic component behind CASA values and SCR.

Since the genetic correlations between CASA values and SCR are so low we were unable to use this study's CASA values for genetic prediction of fertility. We also found a basic genetic component to the CASA values, but the residual effects had a significant influence, making it difficult to estimate future performance of these bulls' fertility. The highest genetic correlation was between BCF and SCR (0.1413). Financially, it would cost approximately $5 per bull to run CASA on every bull. In research the use of CASA is ideal to possibly predict fertility or to utilize as a tool for assessing semen quality. But currently there are cheaper and more accurate methods of predicting fertility for the industry to use in the selection of future sires, such as scrotal circumference EPDs and BSEs. If more research was done into figuring out how to decrease the variance of the

physiological process of sperm creation, or conversely properly account for that variance in a genetic model, the ability to predict fertility would be greater.

Computer assisted semen analysis is a powerful tool utilized in the animal research field, but since CASA traits exhibit low heritability, using CASA to select for future fertility is of limited worth without further investigation of the genetics behind this physiological process. The current BSEs exhibit more heritability than CASA parameters and work effectively for selecting sound breeders. In this study CASA unfortunately is not a good supplementation to BSEs, for sire selection. Currently the industry selects based on genetics, e.g. milk production, and a sire may have great genetics to choose from but could lack the ability to create viable sperm, or even have decreased fertility. This is where CASA has the ability to aid genetic selection; it could also be utilized as a marketing tool to promote a great motility or good cell conformation in bulls.

## LITERATURE CITED


Ansah, G. A., J. C. Segura, and R. B. Buckland. 1985. Semen production, sperm quality, and their heritabilities as influenced by selection for fertility of frozen-thawed semen in the chicken. Poult. Sci. 64:1801-1803.

Ansah, G. A. and R. B. Buckland. 1983. Eight generations of selection for duration of fertility of frozen-thawed semen in the chicken. Poult. Sci. 62:1529-1538.

Bailey, J. L., L. Robertson, M. M. Buhr. 1994. Relations among *in vivo* fertility, computer-analysed motility and $Ca^{++}$ influx in bovine spermatozoa. Can. J. Anim. Sci. 74:53-58.

Beef Improvement Federation. 2010. Guidelines for Uniform Beef Improvement Programs. 9th Ed.. Raleigh, NC.

Bourdon, R. M. and J. S. Brinks. 1986. Scrotal circumference in yearling Hereford bulls: Adjustment factors, heritabilities and genetic, environmental and phenotypic relationships with growth traits. J. Anim. Sci. 62:958-967.

Branton, C., C. B. James, T. E. Patrick and M. H. Newsom. 1951. The relationship between certain semen quality tests and fertility and the interrelationship of these tests. J. Dairy Sci. 34:310-316.

Bratton, R. W., R. H. Foote, C. R. Henderson, S. D. Musgrave, R. S. Dunbar, Jr., H. O. Dunn, and J. P. Beardsley. 1956. The relative usefulness of combinations of laboratory tests for predicting the fertility of bovine semen. J. Dairy Sci. 39:1542-1549.

Brun, J. M., A. Sanchez, R. Duzert, G. Saleil and M. Theau-Clément. 2010. Genetic parameters of rabbit semen traits. 9th World Congr. Genet. Appl. Livest. Prod. Leipzig, Germany. August 1–6, 2010.

Bruner, K. A., R. L. McCraw, M. D. Whitacre and S. D. Van Camp. 1995. Breeding soundness examination of 1,952 yearling beef bulls in north carolina. Theriogenology. 44:129-145.

Buckner, P. J., E. L. Willett and N. Bayley. 1954. Laboratory tests, singly and in combination, for evaluating fertility of semen and of bulls. J. Dairy Sci. 37:1050-1060.

Budworth, P.R. 1987. Computerized methods to measure motion of bull spermatozoa and correlations with fertility. MS Thesis. Colorado State Univ., Fort Collins.

Budworth, P. R., R. P. Amann, and P. L. Chapman. 1988. Relationships between computerized measurements of motion of frozen-thawed bull spermatozoa and fertility. J. Androl. 9:41-54.

Budworth, P. R., R. P. Amann, and R. H. Hammerstedt. 1987. A microcomputer-photographic method for evaluation of motility and velocity of bull sperm. J. Dairy Sci. 70:1927-1936.

Chacón, J. 2001. Assessment of sperm morphology in Zebu bulls, under field conditions in the tropics. Reprod. Dom. Anim. 36:91-99.

Chenoweth, P.J. 1983. Sexual behavior of the bull: a review. J. Dairy Sci. 66:173.

Chenoweth, P. J., J. C. Spitzer, and F. M. Hopkins. 1992. A new breeding soundness evaluation form. Proc Soc. For Theriogenology. Annual Mtg. San Antonio, Tx.

Christensen, P., P. B. Brockhoff and H. Lehn-Jensen. 1999. The relationship between semen quality and the nonreturn rate of bulls. Reprod. Dom. Anim. 34:503-507.

Clay, J. S. and B. T. McDaniel. 2001. Computing mating bull fertility from DHI nonreturn data. J. Dairy Sci. 84:1238-1245.

Coe, P. H. and C. D. Gibson. 1993. Adjusted 200-day scrotal size as a predictor of 365-day scrotal circumference. Theriogenology. 40:1065-1072.

Coulter, G. H. and G. C. Kozub. 1989. Efficacy of methods used to test fertility of beef bulls used for multiple-sire breeding under range conditions. J. Anim. Sci. 67:1757-1766.

Coulter, G. H., and R. H. Foote. 1979. Bovine testicular measurements as indicators of reproductive performance and their relationship to productive traits in cattle: a review. Theriogenology. 11:297-311.

Coulter, G. H., T. R. Rounsaville and R. H. Foote. 1976. Heritability of testicular size and consistency in Holstein bulls. J. Anim. Sci. 43:9-12.

Davis, R. O. and D. F. Katz. 1996. Computer-aided sperm analysis: a critical review. Page 30 in Evaluation and Treatment of the Infertile Male. G. M. Centola and K. A. Ginsburg, ed. Cambridge University Press, Cambridge, United Kingdom.

Decker, J. E., P. Luna-Nevarez, A. M. Encinias, R. M. Enns, and M. G. Thomas. 2008. Case study: Scrotal circumference in beef bulls – Predictions of measures at 365 days of age from measures at 240 days of age with data from the Tucumcari bull test. Pro. Anim. Sci. 24:488-493.

Ericsson, S. A., D. L. Garner, C. A. Thomas, T. W. Downing and C. E. Marshall.1993. Interrelationships among fluorometric analyses of spermatozoal function, classical semen quality parameters and the fertility of frozen-thawed bovine spermatozoa. Theriogenology. 39:1009-1024.

Farin, P. W. 1980. Sexual behavior and fertility in beef bulls. MS Thesis. Colorado State Univ., Fort Collins.

Farrell, P. B., G. A. Presicce, C. C. Brockett and R. H. Foote. 1998. Quantification of bull sperm characteristics measured by computer-assisted sperm analysis (CASA) and the relationship to fertility. Theriogenology. 49:871-879.

Foote, R. H. 1970. Inheritance of fertility – facts, opinions, and speculations. J. Dairy Sci. 53:936-944.

Gilmour, A.R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2006. ASReml user guide release 2.0. VSN International Ltd, Hemel Hempstead, HP1 1ES, UK

Graham, E. F., M. K. L. Schmehl and D. S. Nelson. 1980. Problems with laboratory assays. Page 59 in Proc. 8th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia, MO.

Graham, J. K., E. Kunze and R. H. Hammerstedt. 1990. Analysis of sperm cell viability, acrosomal integrity, and mitochondrial function using flow cytometry. Biol. Reprod. 43:55-64.

Gunsett, F. C. 1984. Linear index selection to improve traits defined as ratios. J. Anim. Sci. 59: 1185-1193.

Hamilton Thorne Research. Operation manual. Beverly, MA, USA.

Hicks, C. T. Dyer. 2010. Selecting a beef breed. http://www.caes.uga.edu/applications/publications/files/pdf/C%20859_2.PDF. Accessed April 20, 2010.

Hoflack, G., G. Opsomer, T. Rijsselaer, A. Van Soom, D. Maes, A. de Kruif and L. Duchateau. 2007. Comparison of computer-assisted sperm motility analysis parameters in semen from Belgian Blue and Holstein-Friesian bulls. Reprod. Dom. Anim. 42:153-161.

Hopkins, F. M., DVM, and J. C. Spitzer. 1997. The new society for theriogenology breeding soundness evaluation system. Page 283-293 in: The Veterinary Clinics of North America: Food Animal Practice: Bull Infertility. vol 13 num 2. E. Hunt, DVM, S. D. Van Camp, DVM, ed. W. B. Saunders Company, Philadelphia, PA.

Januskauskas, A., A. Johannisson and H. Rodríguez-Martínez. 2003. Subtle membrane changes in cryopreserved bull semen in relation to sperm viability, chromatin structure and field fertility. Theriogenology. 60:743-758.

Kasimanickam, R., R. L. Nebel, J. D. Peeler, W. L. Silvia, K. T. Wolf, A. J. McAllister and B. G. Cassell. 2006. Breed differences in competitive indices of Holstein and Jersey bulls and their association with sperm DNA fragmentation index and plasma membrane integrity. Theriogenology. 66:1307-1315.

Kaskar, K. and D. R. Franken. 1996. Evaluation of sperm motility in assisted reproduction. Page 73 in Evaluation and Treatment of the Infertile Male. G. M. Centola and K. A. Ginsburg, ed. Cambridge University Press, Cambridge, United Kingdom.

Kastelic, J. P. and J. C. Thundathil. 2008. Breeding soundness evaluation and semen analysis for predicting bull fertility. Reprod. Dom. Anim. 43(Suppl. 2):368-373.

Kealey, C. G., M. D. MacNeil, M. W. Tess, T. W. Geary, and R. A. Bellows. 2004. Genetic parameter estimates for yearling scrotal circumference and semen traits of line 1 Hereford bulls. Proc. West. Sec. Amer. Soc. Anim. Sci. 55:36-39.

Kennedy, S. P., J. C. Spitzer, F. M. Hopkins, H. L. Higdon III, W. C. Bridges Jr.. 2002. Breeding soundness evaluations of 3648 yearling beef bulls using the 1993 society for theriogenology guidelines. Theriogenology. 58:947-961.

Kjaestad, H., E. Ropstad and K. Andersen Berg. 1993. Evaluation of spermatological parameters used to predict the fertility of frozen bull semen. Acta Vet. Scand. 34:299-303.

Knights, S. A., R. L. Baker, D. Gianola and J. B. Gibb. 1984. Estimates of heritabilities and of genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. J. Anim. Sci. 58:887-893.

Kruger, T. F. and T. C. du Toit. 1996. Evaluation of sperm morphology by computer analysis. Pages 109-115 in Human Spermatozoa in Assisted Reproduction. A. A. Acosta and T. F. Kruger ed. The Parthenon Publishing Group, Pearl River, NY.

Kuhn, M. T. and J. L. Hutchinson. 2008. Prediction of dairy bull fertility from field data: Use of multiple services and identification and utilization of factors affecting bull fertility. J. Dairy Sci. 91:2481-2492.

Kuhn, M. T., J. L. Hutchinson and H. D. Norman. 2008. Modeling nuisance variables for prediction of service sire fertility. J. Dairy Sci. 91:2823-2835.

Larson, L. L. 1986. Examination of the reproductive system of the bull. Pages 101-116 in Current Therapy in Theriogenology 2. D. A. Morrow, ed. W. B. Saunders Company, Philadelphia.

Latimer, F. G., L. L. Wilson, M. F. Cain and W. R. Stricklin. 1982. Scrotal measurements in beef bulls: Heritability estimates, breed and test station effects. J. Anim. Sci. 54:473-479.

Lavara, R., A. Moreno, J. S. Vicente and M. Baselga. 2010. Genetic parameters of sperm subpopulations in a paternal rabbit line. 9th World Congr. Genet. Appl. Livest. Prod. Leipzig, Germany. August 1–6, 2010.

Linford, E., F. A. Glover, C. Bishop and D. L. Stewart. 1976. The relationship between semen evaluation methods and fertility in the bulls. J. Reprod. Fertil. 47:283.

Madrid, N., R. S. Ott, D. N. Rao Veeramachaneni, D. F. Parrett, W. Vanderwert and C. L. Willms.1988. Scrotal circumference, seminal characteristics, and testicular lesions of yearling angus bulls. Am. J. Vet. Res. 49:579-585.

Mahmoud, A. M. A., S. Gordts, A. Vereecken, A. Serneels, R. Campo, L. Rombauts and F. H. Comhaire. 1998. Performance of the sperm quality analyser in predicting the outcome of assisted reproduction. Intl. J. Androl. 21:41-46.

Makarechian M. and A. Farid. 1985. The relationship between breeding soundness evaluation and fertility of beef bulls under group mating at pasture. Theriogenology. 23:887-898.

Marks, H. L. 1978. Possible relationship between packed sperm volume and egg mass in domestic fowl. Experentia. 34:443-444

Mortimer, D. 1994. Computer-aided sperm analysis and sperm kinematics. Pages 135-157 in Practical Laboratory Andrology. Oxford University Press, New York, NY.

Mosé, E. and J. K. Graham. 2008. In vitro evaluation of sperm quality. Anim. Reprod. Sci.. 105:104-118.

Mukasa-Mugerwa, E. 1974. Genetics of bovine spermatozoan morphology. PhD Diss. Colorado State Univ., Fort Collins.

Mukhopadhyay, C. S., A. K. Gupta, B. R. Yadav, L. S. Chauhan, A. Gupta, T. K. Mohanty and V. S. Raina. 2011. Effect of crypreservation on sperm chromatin integrity and fertilizing potential in bovine semen. Liv. Sci. 136:114-121.

Nestor, K. E. 1977. The influence of genetic change in egg production, body weight, fertility or response to cold stress on semen yield in the turkey. Poultry Sci. 56:421-425.

Norman, H. D., J. L. Hutchison and J. R. Wright. 2008. Sire conception rate: New national AI bull fertility evaluation. http://www.aipl.arsusda.gov/reference/arr-scr1.htm. Accessed July 30, 2009.

O'Connor, M. T., R. P. Amann and R. G. Saacke. 1981. Comparisons of computer evaluations of spermatoxoal motility with standard laboratory tests and their use for predicting fertility. J. Anim. Sci. 53:1368-1376.

Ott, R. S. 1986. Breeding soundness examination in bulls. Pages 125-136 in Current Therapy in Theriogenology 2. D. A. Morrow, ed. W. B. Saunders Company, Philadelphia.

Palmer, C. W. and A. D. Barth. 2003. Comparison of the BullMate sperm quality analyzer with conventional means of assessing the semen quality and breeding soundness of beef bulls. Anim. Reprod. Sci. 77:173-185.

Rodríguez-Martínez, H. 2003. Laboratory semen assessment and prediction of fertility: Still Utopia?. Reprod. Dom. Anim. 38:312-318.

Rodríguez-Martínez, H. 2006. Can We Increase the Estimative Value of Semen Assessment?. Reprod. Dom. Anim. 41(Suppl. 2):2-10.

Rodríguez-Martínez, H. 2007. State of the art in farm animal sperm evaluation. Reprod. Fertil. Dev. 19:91-101.

Saacke, R. G. 1982. Components of semen quality. J. Anim. Sci. 55:Suppl 2:1-13.

Saacke, R. G. 2004. Sperm morphology. Pages 67-71 in Proc. Annu. Conference Am. Assoc. Bov. Prac. Fort Worth, TX.

Saacke, R. G., W. E. Vinson, M. L. O'Connor, J. E. Chandler, J. Mullins, R. P. Amann, C. E. Marshall, R. A. Wallace, W. N. Vincel and H. C. Kellgren. 1980. The relationship of semen quality and fertility; A heterospermic study. Page 75 in Proc. 8th Tech. Conf. Artif. Insem. Reprod., Natl. Assoc. Anim. Breeders, Columbia, MO.

Segura, J. C., J. S. Gavora, R. A. Fairfull, R. S. Gowe, and R. B. Buckland. 1990. Heritability estimates of male reproductive and morphological traits and their genetic correlations with female egg production and other related traits in chicken. Poult. Sci. 69:493-501.

Smith, B. A., J. S. Brinks and G. V. Richardson. 1989. Estimation of genetic parameters among breeding soundness examination components and growth traits in yearling bulls. J. Anim. Sci. 67:2892-2896.

Sosa, J. M., P. L. Senger and J. J. Reeves. 2002. Evaluation of American Wagyu sires for scrotal circumference by age and body weight. J. Anim. Sci. 80:19-22.

Stålhammar, E. M., L. Janson and J. Philpsson. 1994. The impact of sperm motility on non-return rate in preselected dairy bulls. Reprod. Nutr. Dev. 34:37-45.

Swanson, E. W. and H. A. Herman. 1944. The correlation between some characteristics of dairy bull semen and conception rate. J. Dairy Sci. 27:297-301.

Trenkle, A. and R. L. Willham. 1977. Beef production efficiency. Sci. 198:1009-1015.

USDA-ARS-AIPL. 2010. Description of national phenotypic evaluation systems. Accessed Sept 1, 2011. http://aipl.arsusda.gov/reference/Form_PE_MFertility-SCR_1008.pdf.

Weigel, K. A. and S. W. Lin. 2002. Controlling inbreeding by constraining the average relationship between parents of young bulls entering AI progeny test programs. J. Dairy Sci. 85:2376-2383.

Zavos, P. M., J. R. Correa and P. N. Zarmakoupis-Zavos. 1996. Measurement of the sperm motility index via the sperm quality analyzer and its relationship to other qualitative sperm parameters. Theriogenology. 46:421-427.

# APPENDIX A

## CODE USED IN ANALYSIS

*SAS Code*

```
proc import
     datafile='C:\Users\Mandy\Documents\SAS_Data\DBSwithSCR
     .csv' out=holsteindat3 dbms=csv replace;
     getnames=yes;
run;
proc univariate data=holsteindat3 plot normal alpha=0.05;
     var MOTILE_PCT PROGRESSIVE_PCT;
run;
proc import
     datafile='C:\Users\Mandy\Documents\SAS_Data\DBT_full_S
     CR_full.csv' out=holsteindat dbms=csv replace;
     getnames=yes;
run;
data holsteindat2;
     set holsteindat;
          lVAP=log10(VAP);
          lVCL=log10(VCL);
          ALH1=SQRT(ALH);
          iSIZE=1/SIZE;
          SIZE2=SIZE*SIZE;
          SIZE3=SIZE*SIZE*SIZE;
run;
proc corr;
     var VAP VSL VCL;
run;
proc univariate data=holsteindat2 plot normal alpha=0.05;
     var VAP VSL VCL ALH BCF STR LIN SIZE lVAP ALH1 iSIZE
     SCR;
run;
proc glm data=holsteindat2 alpha=0.05;
     class Semen_comp ID;
     model SIZE = Semen_comp ID /solution;
     means ID / REGWQ SNK;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
```

```
     model VAP = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model VSL = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model VCL = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model ALH = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model BCF = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model SIZE = Semen_comp /solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model lVAP = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
```

```
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model ALH1 = Semen_comp SIZE3/solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
proc mixed data=holsteindat2 covtest;
     class Semen_comp ID;
     model iSIZE = Semen_comp /solution;
     random intercept / subject=ID(Semen_comp) type=un
     solution;
     lsmeans Semen_comp /pdiff cl;
run;
```

*Animal Breeders Toolkit Code*

```
##DBT_full_remove.csv header
##FIELD#,TRACK#,DATE,TIME,TRACK_TYPE,POINTS,VAP,LVAP,VSL,VC
L,ALH,SQALH,BCF,STR,LIN,ELONGATION,SIZE,ISIZE,SIZE_PIXELS,I
NTENSITY,NAME,ID,Regnum,SCR,ISCR,Semen_comp,SSCR

awk 'BEGIN{FS=","}; NR>1 {print
$7,$11,$13,$17,$22,$23,$24,$26}' DBT_full_remove.csv >
DBT_un.dat

############################################################
##DBT_un.dat header
##VAP ALH BCF SIZE ID Regnum SCR Semen_comp

par_ped_stk.txt is stacked

#All unknown parents need to be recoded to .
awk '{for(i=1;i<=NF;i++) if($i=="0") {$i="."}} {print
$1,$2,$3}' ped.recode > ped.rc

ainv -i ped.rc -o inbred.lst

awk '{print $2}' inbred.lst | sst

############################################################
##Header:Regnum,TOTAL_COUNT,TOTAL_CONC,MOTILE_PCT,PROGRESSI
VE_PCT,Semen_comp,SCR

awk 'BEGIN{FS=","}; NR>1 {print $1,$9,$10,$11,$12,$14,$15}'
DBSRegnumSC.csv > DBSmot.dat
```

```
#############################################################
##Header:
Track_Type,VAP,lVAP,ALH,SQALH,BCF,SIZE,iSIZE,SIZE3,Regnum,S
emen_comp,SCR

awk 'BEGIN{FS=","}; NR>1 {print
$1,$2,$3,$6,$7,$8,$12,$13,$14,$17,$18,$19}'
DBT_trans_SCR_single.csv > DBTtrans.dat

#############################################################
##DBT_file.csv Header:
##FIELD#,TRACK#,cellnum,DATE,TIME,TRACK_TYPE,POINTS,VAP,VSL
,VCL,ALH,BCF,STR,LIN,ELONGATION,SIZE,SIZEadj,SIZE2,SIZE_PIX
ELS,INTENSITY,NAME,ID,Regnum,Semen_comp,Birthdate

awk 'BEGIN{FS=","}; NR>1 {print
$3,$7,$8,$11,$12,$15,$17,$18,$21,$22,$23,$24}' DBT_file.csv
> DBT

##DBT Header:
##cellnum,POINTS,VAP,ALH,BCF,ELONGATION,SIZEadj,SIZE2,NAME,
ID,
   Regnum,Semen_comp

awk '{print $10}' DBT | sort | uniq -c | awk '{print
$1,$2}' > Id.cnt
##Id.cnt format
###cells,Registration number

sort -k1,1 par_ped_stk.txt > par_ped.stk

join -a2 -j1 1 -j2 2 -e"0" -o 2.2 1.2 1.3 par_ped.stk
Id.cnt > regsd

## have a sire and dam for each of the 120 sires

#############################################################
## how many unique SCR values?
awk 'BEGIN{FS=","}; NR>1 {print $13}' DBT_remove_cells.csv
| sort -u | wc
```

*ASReml Code*

```
Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
```

```
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

VAP ~ mu SIZE3 Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   VAP
   ALH
   BCF
   SIZE
   ID
   Regnum 120 !P !LL 17
   SCR
   Semen_comp 4 !A
   SSCR

stack_cor.ped !ALPHA !MAKE
DBT_un.dat !MVINCLUDE !MAXIT 400

ALH ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 100
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   VAP
   ALH
   BCF
   SIZE
   ID
```

```
   Regnum 120 !P !LL 17
   SCR
   Semen_comp 4 !A
   SSCR

stack_cor.ped !ALPHA !MAKE
DBT_un.dat !MVINCLUDE !MAXIT 400

BCF ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 100
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

SIZE ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV

DBS Holstein SCR 2010
   Regnum 120 !P !LL 17
   TOTAL_COUNT
   TOTAL_CONC
   MOTILE_PCT
   PROGRESSIVE_PCT
   Semen_comp 4 !A
```

```
stack_cor.ped !ALPHA !MAKE
DBSmot.dat !MVINCLUDE !MAXIT 400 !STEP 0.01 !EXTRA 10

MOTILE_PCT ~ mu Semen_comp !r Regnum

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

lVAP ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR
```

```
stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

SQALH ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

iSIZE ~ mu Semen_comp !r Regnum ide(Regnum)

0 0 1
Regnum 2
1 0 US 2
Regnum 0 AINV


Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
```

```
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

VAP SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
Regnum

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

ALH SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
```

```
Regnum

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

BCF SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
Regnum

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
```

```
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

SIZE SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
Regnum

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

lVAP SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
Regnum

Holstein Data Pepper Thesis 2009
   Track_Type 3 !A
```

```
   VAP
   lVAP
   ALH
   SQALH
   BCF
   SIZE
   iSIZE
   SIZE3
   Regnum 120 !P !LL 17
   Semen_comp 3 !A
   SCR

stack_cor.ped !ALPHA !MAKE
DBTtrans.dat !MVINCLUDE !MAXIT 4000 !STEP 0.01 !EXTRA 10

SQALH SCR ~ Trait Trait.Semen_comp !r Trait.Regnum

1 2 1
0
Trait 0 US
0.88
0.6371 1
Trait.Regnum
2 0 US
0.08
0.0022 1
Regnum
```

*Estimating heritability, and correlations (genetic, phenotypic and residual)*

For single trait model:

```
F Vp 1+2+3     #4
F Vd 3*1  #5
F Vr 2*1  #6
F Ve 1*1  #7
F AE 3+1        #8
H H2d 5 4 #9
H C2 7 4  #10
H R 8 4         #11
```

For multivariate model:

```
F Vp 1:3 + 4:6  #7,8,9
F Vd 4:6 * 1    #10,11,12
H H2dA 10 7
```

```
H H2dS 12 9
R Rg 4:6
R Rr 1:3
R Rp 7 8 9
```